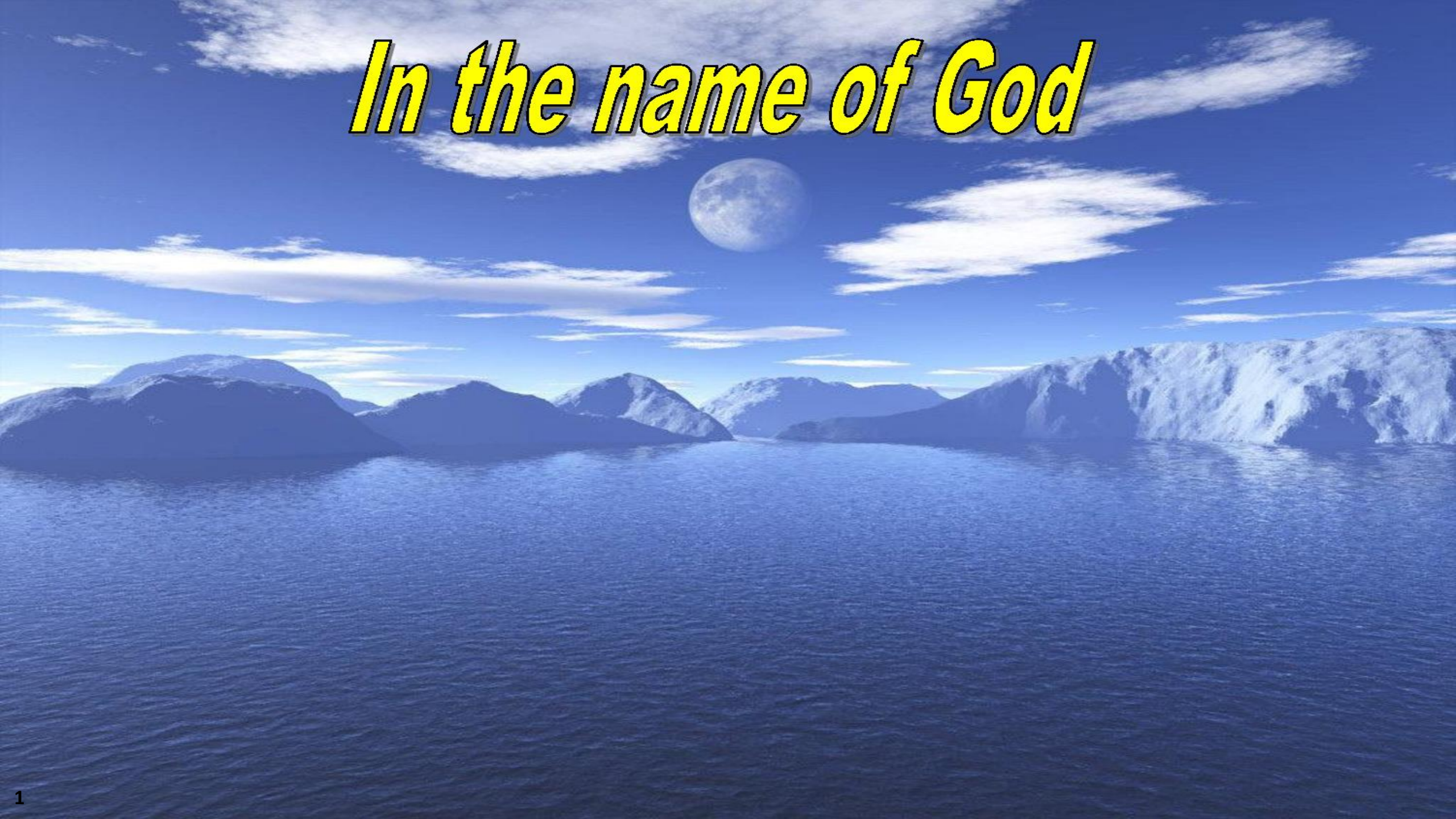

In the name of God

vectors in gene therapy

Presented by : Sahar khojasteh pour

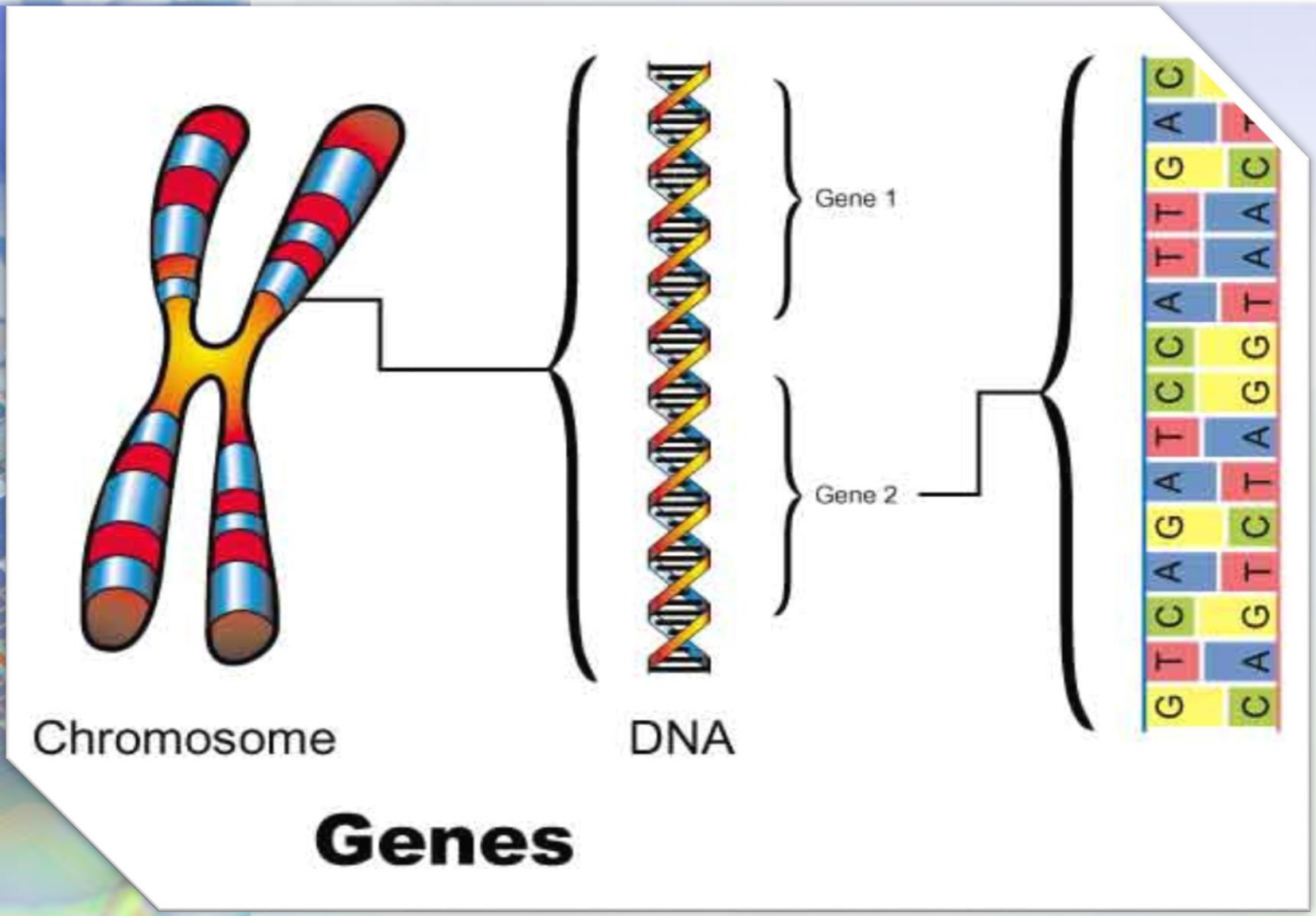
Under supervision of : Dr. Ahmadpour

30 may 2016

Medical of Biotechnology group
Qazvin University medical Sciences

Contents

Genes



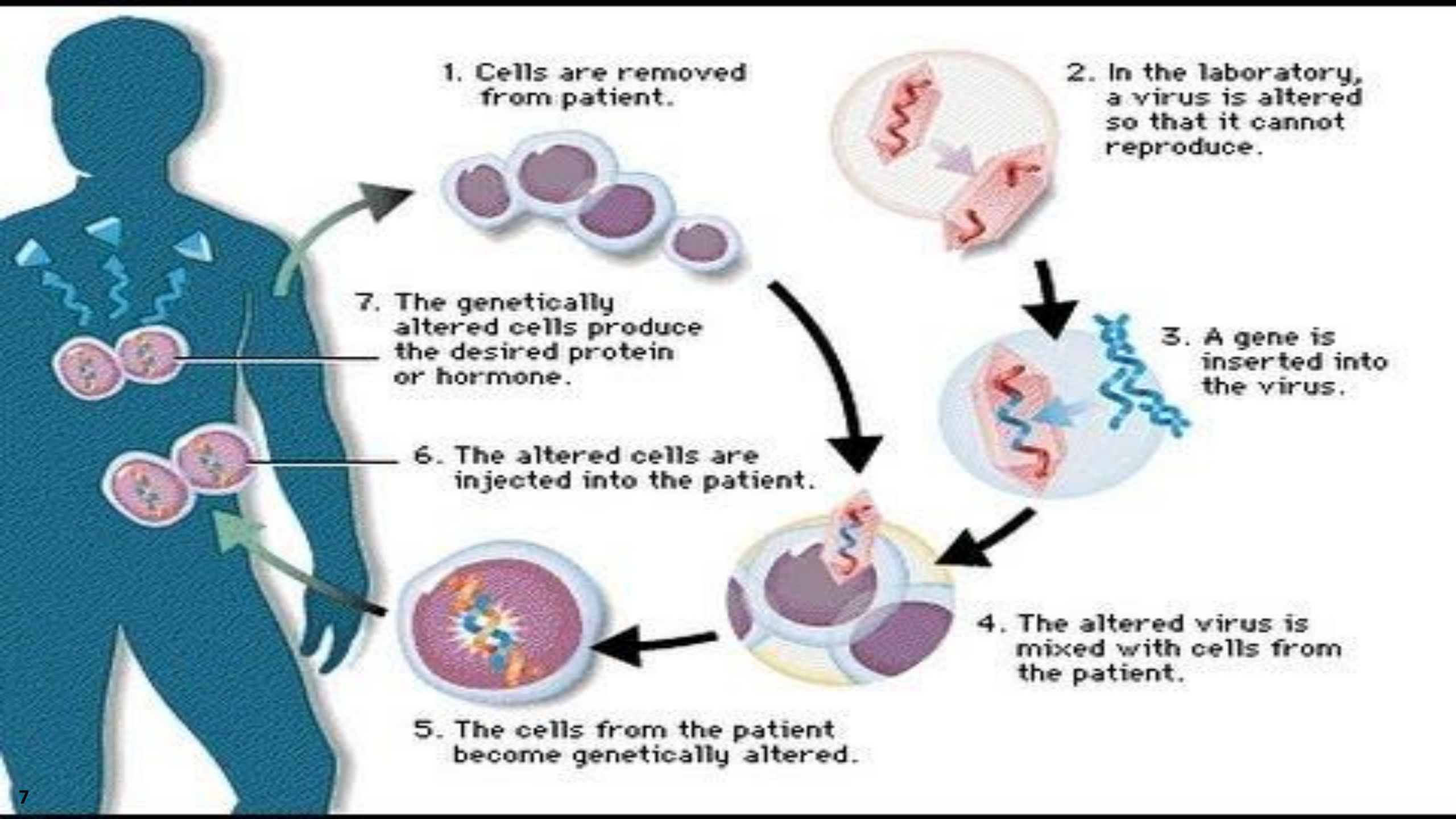
What is Gene Therapy?

- A technique for correcting defective.(1)
- A normal gene inserted to compensate for a nonfunctional gene.(1)
- There are approaches:(2)

  - An abnormal gene traded for a normal gene
  - An abnormal gene repaired through selective reverse mutation
  - Change the regulation of gene pairs

What is Gene Therapy?

- ✓ treat, cure, or ultimately prevent disease by changing the expression of a person's genes.(3)
- ✓ current gene therapy is primarily experimental, with most human clinical trials only in the research stages.(4)
- ✓ Transfer of genetic material to cure a disease or at least to improve the clinical status.(4)


1. Cells are removed from patient.
2. In the laboratory, a virus is altered so that it cannot reproduce.
3. A gene is inserted into the virus.
4. The altered virus is mixed with cells from the patient.
5. The cells from the patient become genetically altered.
6. The altered cells are injected into the patient.
7. The genetically altered cells produce the desired protein or hormone.

History

- ✓ The origins is the first live attenuated vaccines in the **1950s**.(5)
- ✓ The concept of gene therapy arose during the **1960s** and **1970**s.(5)
- ✓ In **1972**, Theodore Friedmann and Richard Roblin published a paper in Science called "Gene therapy for human genetic disease?".(5)

History

- ✓ The first attempt at modifying human DNA was performed in **1980** by Martin Cline.(6)
- ✓ In **1985**, Anderson and colleague demonstrated how cells from people with ADA deficiency could be modified in tissue culture. (6)
- ✓ The first successful and approved nuclear gene transfer in humans was performed in may **1989** by Dr. Steven Rosenberg.(6)

History

✓ **1990** - The first approved gene therapy clinical trial took place when Ashanthi DeSilva, with ADA-deficient SeveCombined Immunodeficiencyre.(7)

The First gene therapy case was performed on September 14th, 1990.

- Ashanti De Silva was treated for SCID(Sever combined immunodeficiency).
- Doctors removed her white blood cells, inserted the missing gene into the WBC, and then put them back into her blood stream.
- This strengthened her immune system
- This only worked for a few months.

How It Works

- ✓ A **vector** delivers the therapeutic gene.(8)
- ✓ The vector's genetic material is **inserted** into the target cell.(8)
- ✓ **Functional proteins** are created from the therapeutic gene.(8)

Three types of gene therapy:

➢ *Monogenic gene therapy*

- Provides genes to encode for the production of a specific protein
- Cystic fibrosis, Muscular dystrophy.(9)

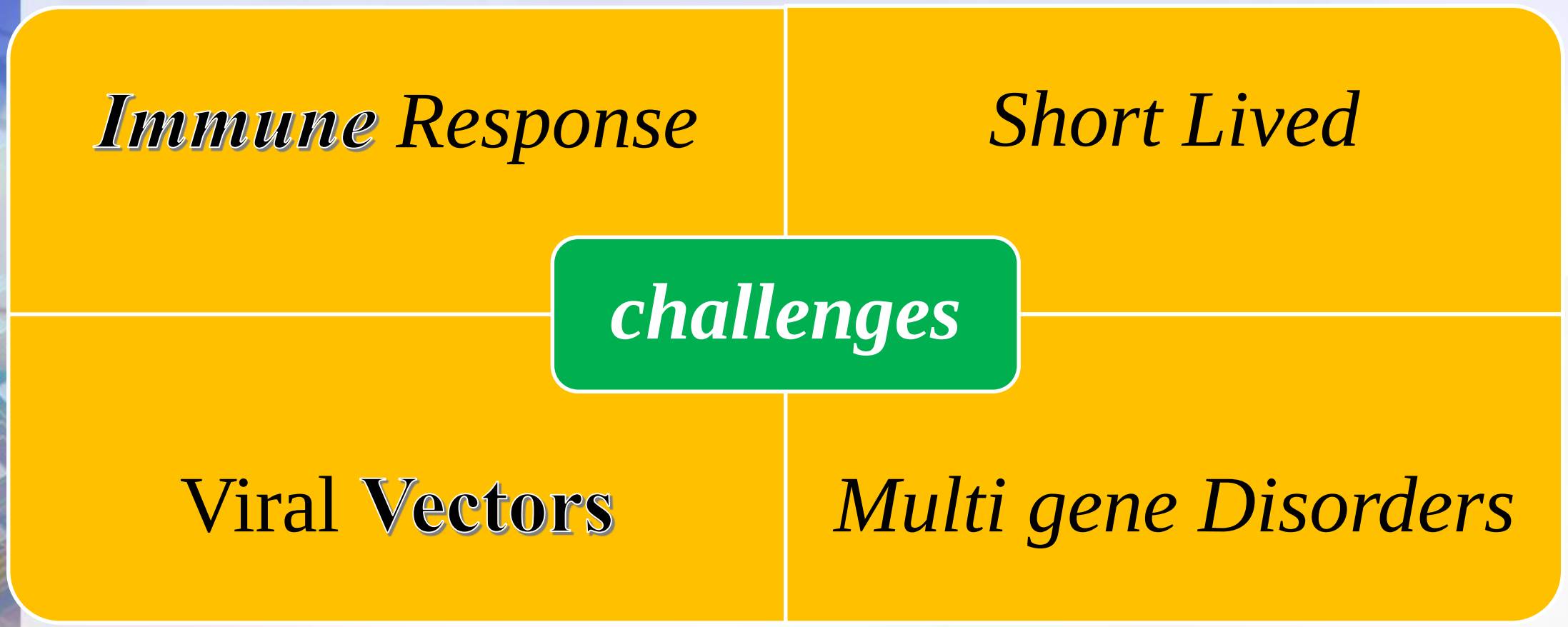
➢ *Suicide gene therapy*

- Provide ‘suicide’ genes to target cancer cells for destruction(9)

➢ *Antisense gene therapy*

- Provides a single stranded gene in an’antisense’ orientation to block the production of harmful proteins.
- AIDS/HIV(9)

challenges in gene therapy(4)



Gene therapy targets

- *Germ line gene therapy*(9)

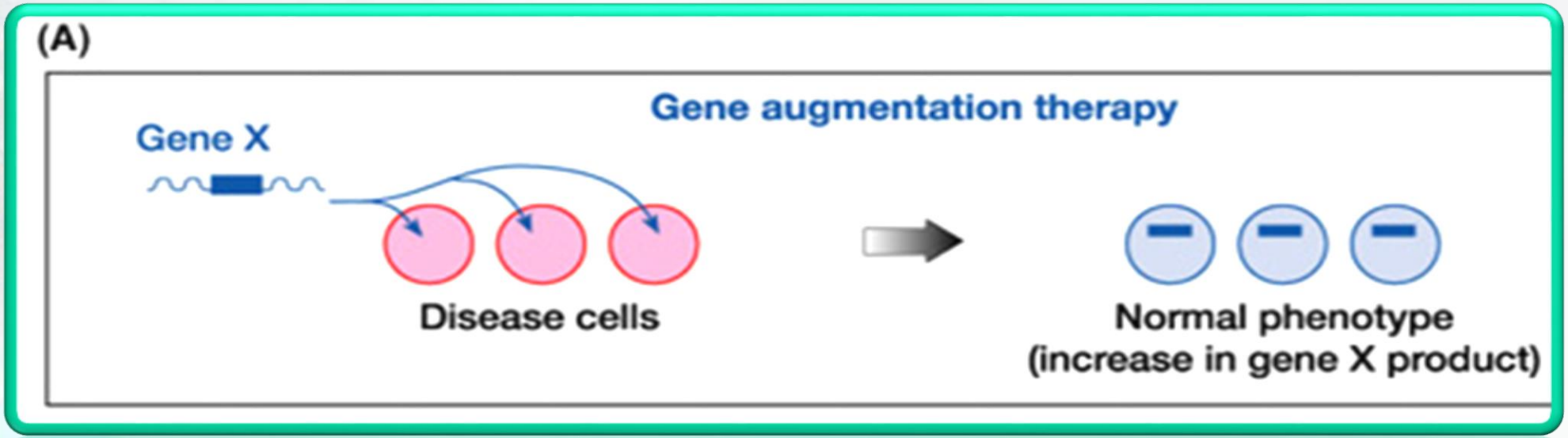
- *Somatic cell gene therapy*(9)

  - Gene augmentation
  - Gene replacement
  - Specific inhibition of gene expression
  - Targeted cell death

Gene therapy targets

➢ *Gene augmentation*

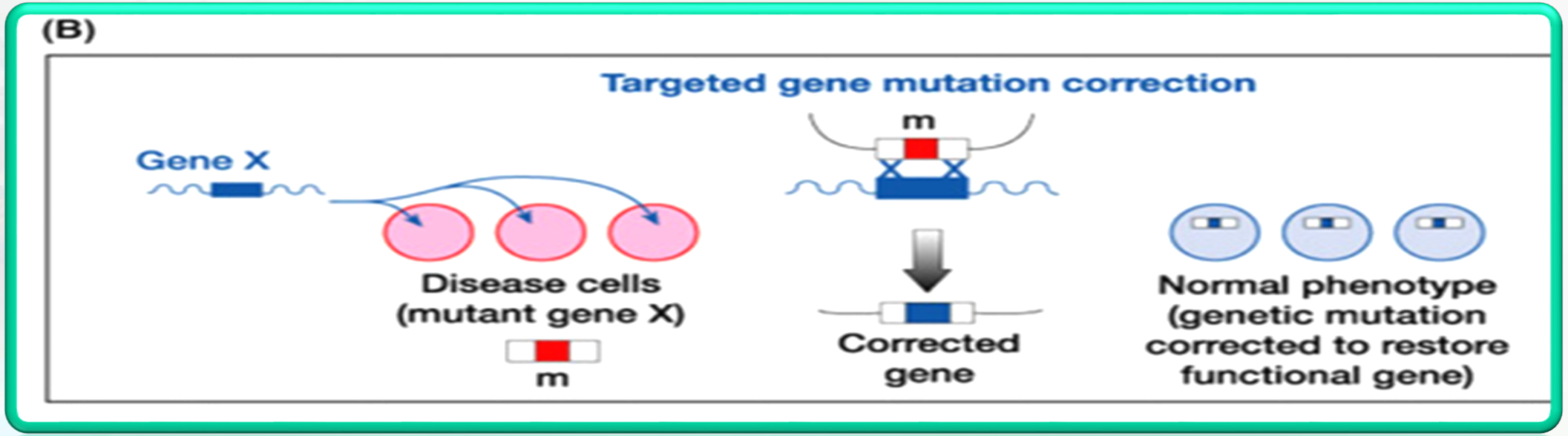
- Add a useful gene into a selected cell type to compensate for the missing or flawed version.(10)
- Useful in treating loss of function mutations.(10)



Gene therapy targets

➢ *Gene replacement*

- Replaces the mutant copy with a correctly functioning copy in situ.(10)
- Useful for gain of function mutations such as oncogenes.(10)



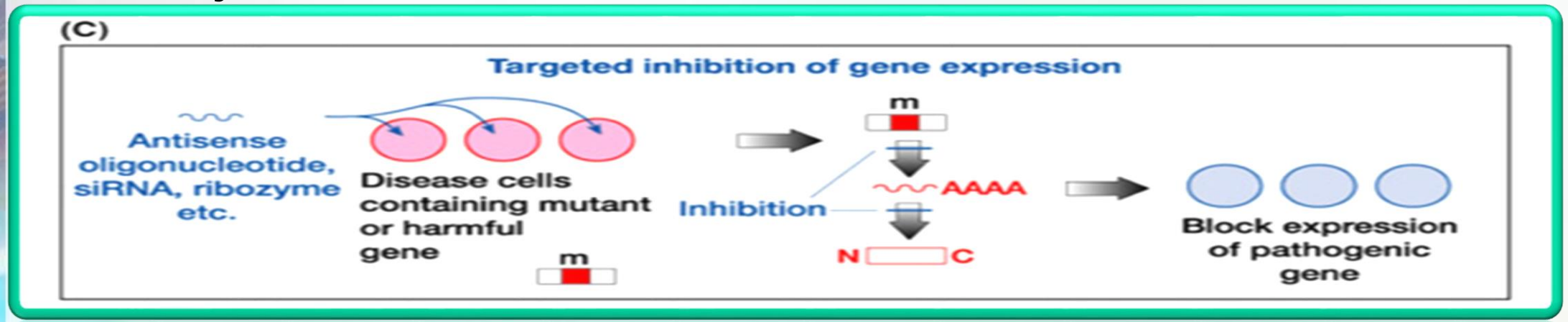
Gene therapy targets

➢ *Specific inhibition of gene expression*

- Involves silencing of specific genes like activated oncogenes.
- Using molecules that degrade RNA transcripts. (10)

✓ *Strategies include*

- **Antisense therapy**
- **siRNA (small interfering RNA)**
- **Ribozymes**



Gene therapy targets

➢ *Targeted cell death*

- Tissue specific toxicity as a result of gene therapy.(10)
- Useful in cancer therapy.(10)

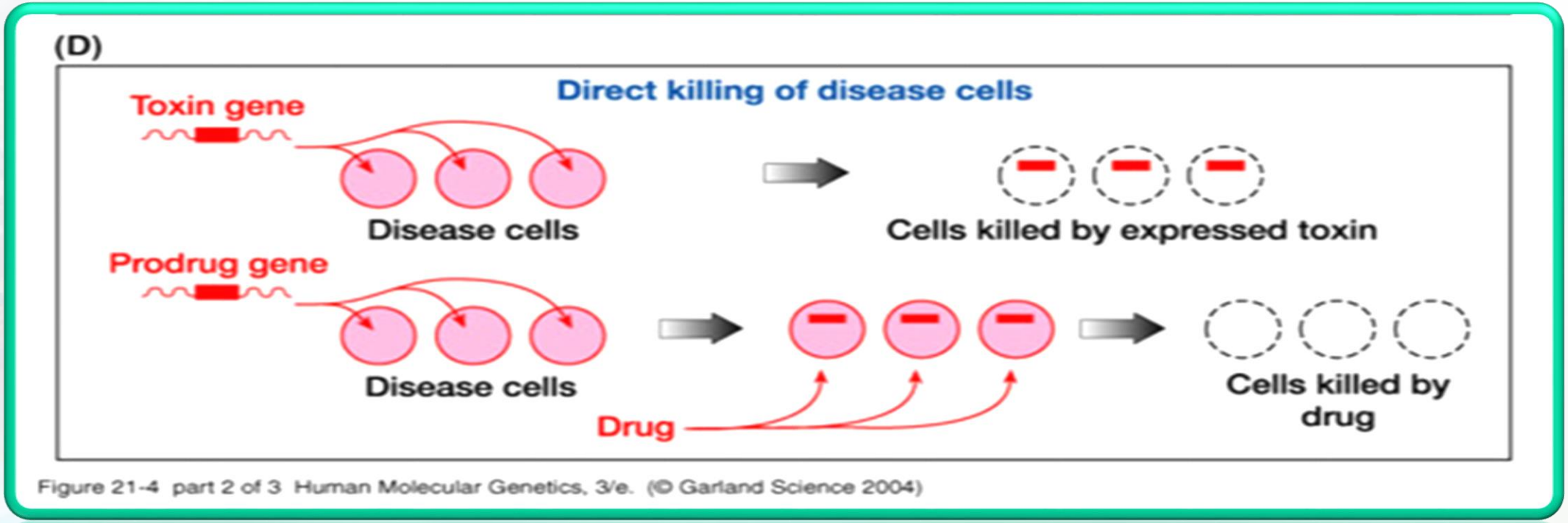


Figure 21-4 part 2 of 3 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Vectors in genetherapy

- ***Vectors*** are carrier molecules which are employed to enhance gene transfer efficiency.(11)

- Ability to **transduce** dividing and non-dividing cells(11)

- Ability to **integrate** into a site-specific location in the host chromosome.(11)

Vectors in genetherapy

➢ *In optimizing a particular vector, one must consider:*

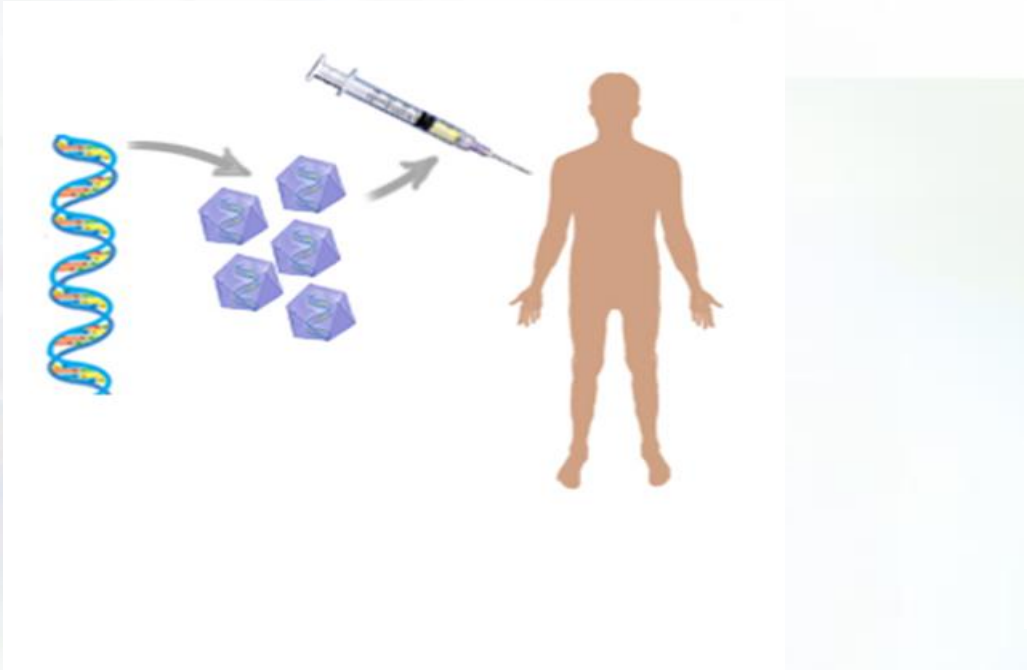
✓ Host immune response(12)

✓ Must target specific tissues for long term gene expression(12)

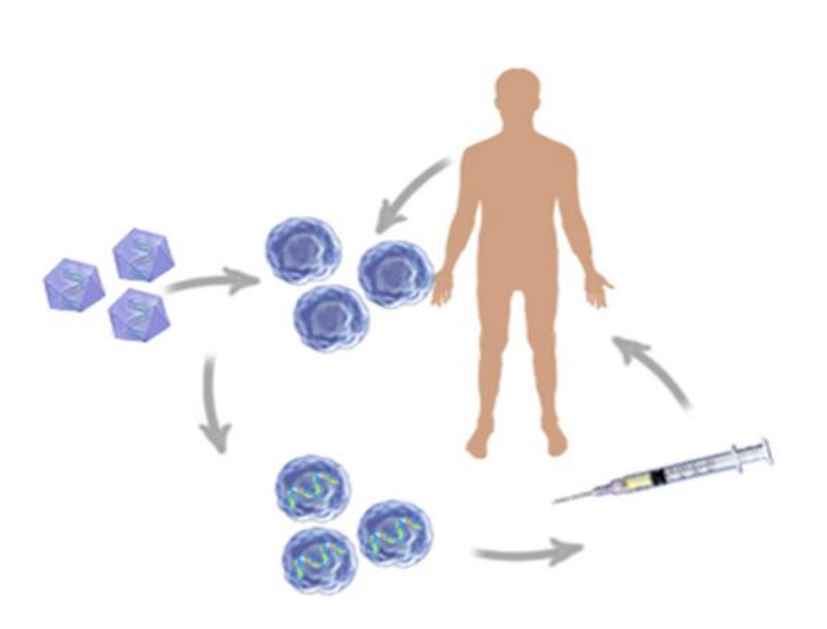
✓ Regulation of the gene after insertion(12)

Strategies for Transgene Delivery

❖ **In vivo Gene Therapy**(4)



❖ **Ex vivo Gene Therapy**(4)



Strategies for Transgene Delivery

❖*In vivo Gene Therapy*

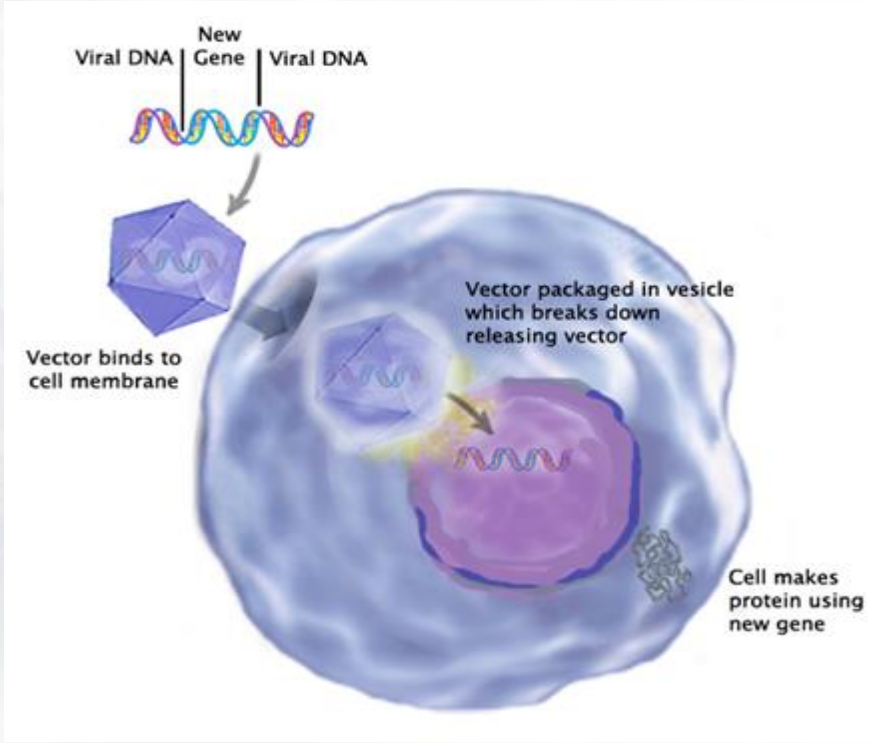
- intravenous or intramuscular or non-invasive.
- Delivery of new genetic material **directly** to target cells within the body.(4)

❖Ex vivo Gene Therapy

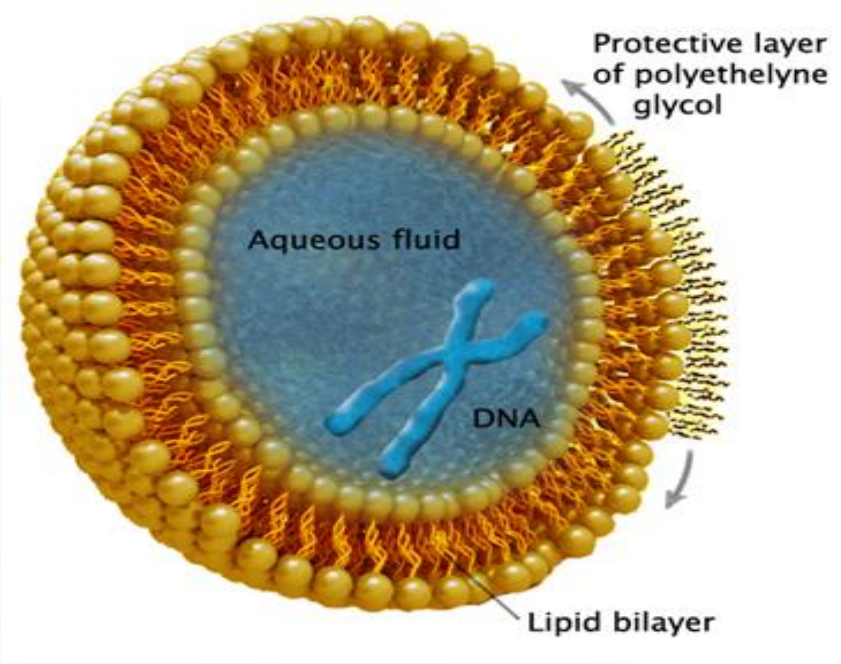
- target cells are **removed** from the body and then genetically modified.(4)
- The cells are then **returned to** the body after selection and amplification(4)
- This is a safe method but dependent on the type of cells being target.

Types of vector

❖Viral



❖ Non-Viral

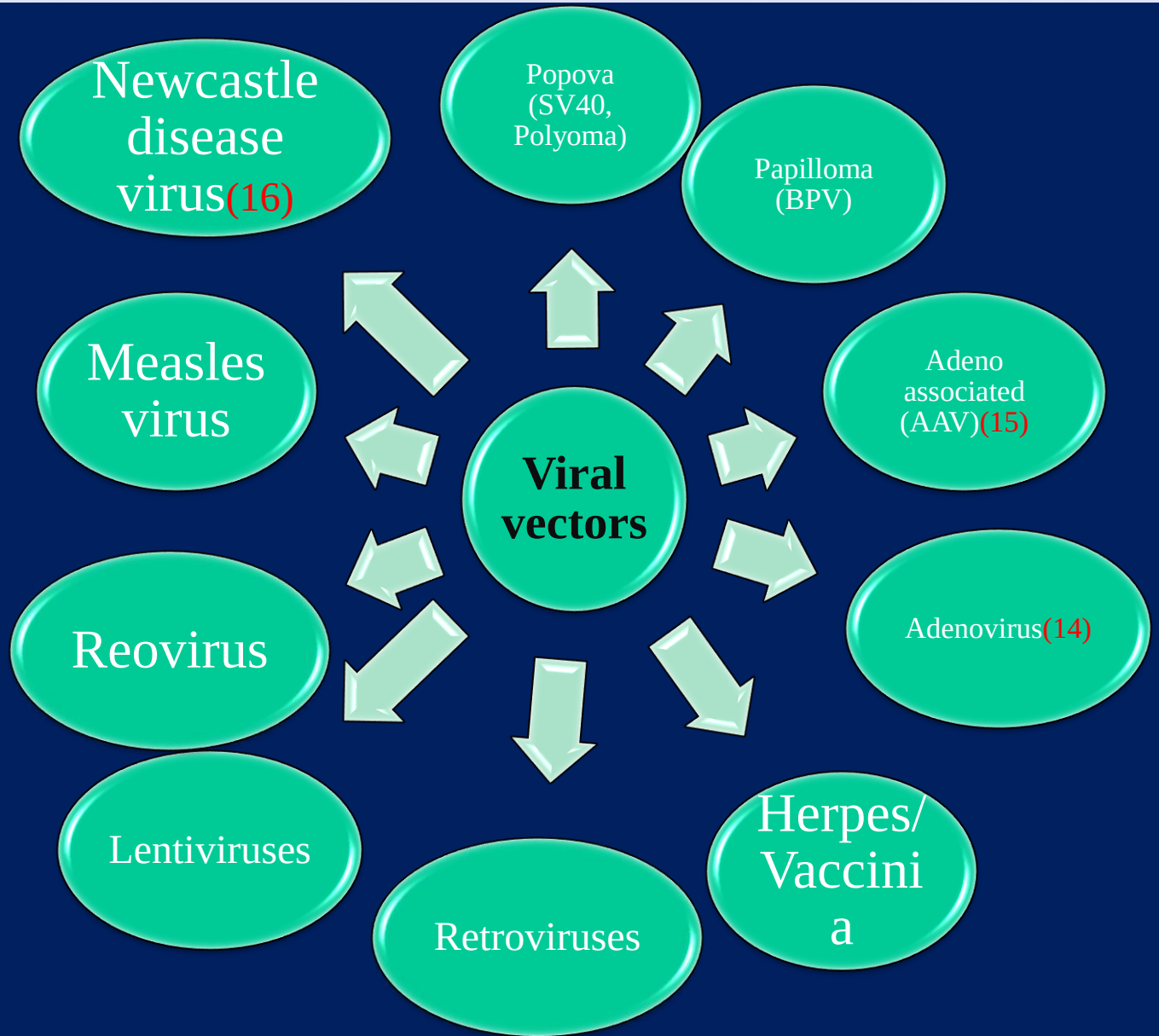


Types of vector

❖*Viral vector*

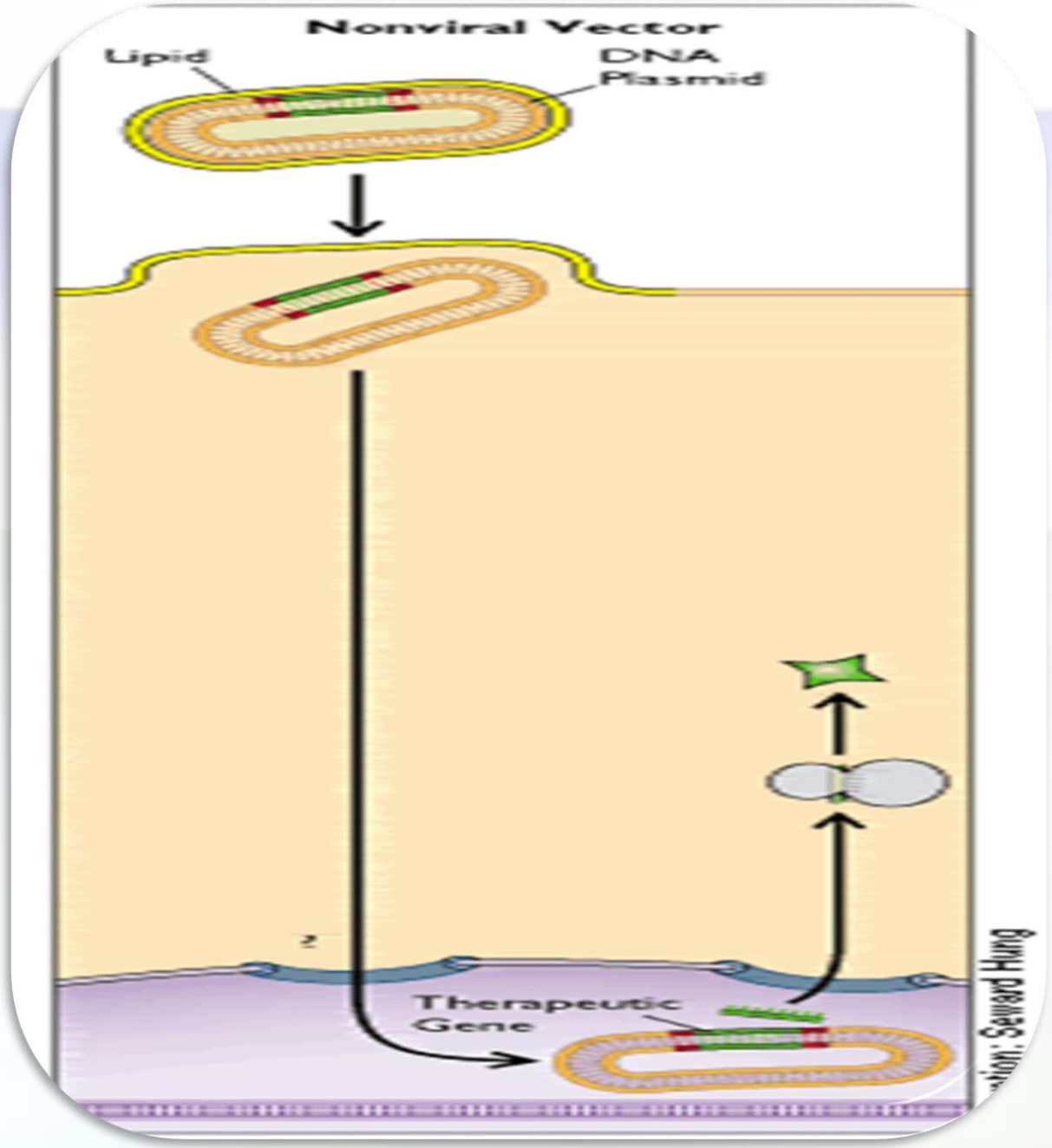
- ✓ Virus particles provide a relatively efficient.(13)
- ✓ Viruses are highly evolved natural vectors for the transfer.(13)
  - ✓ Show some specificity
  - ✓ Immune reaction
  - ✓ Possible infection risk

Types of vector

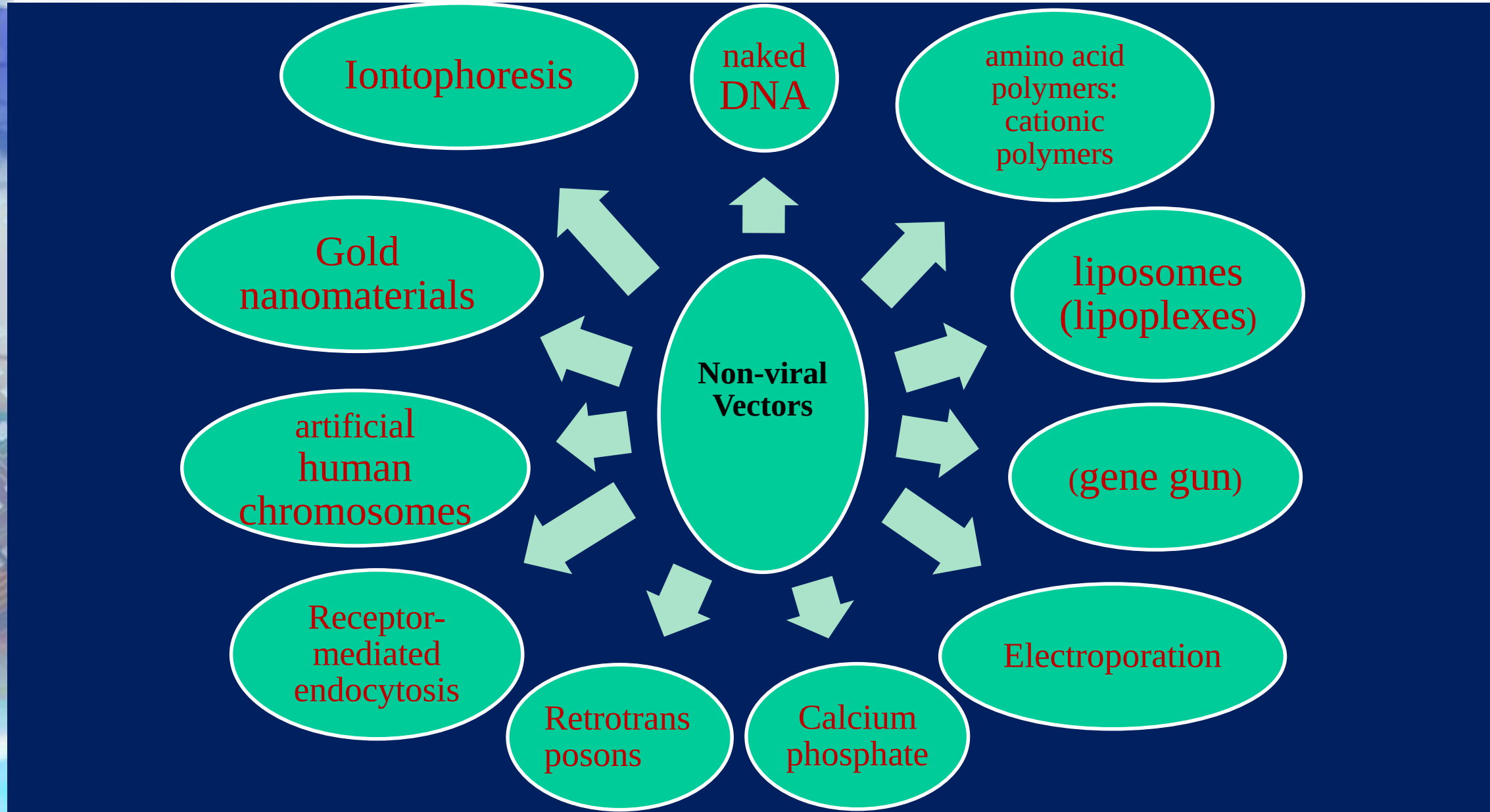


Types of vector

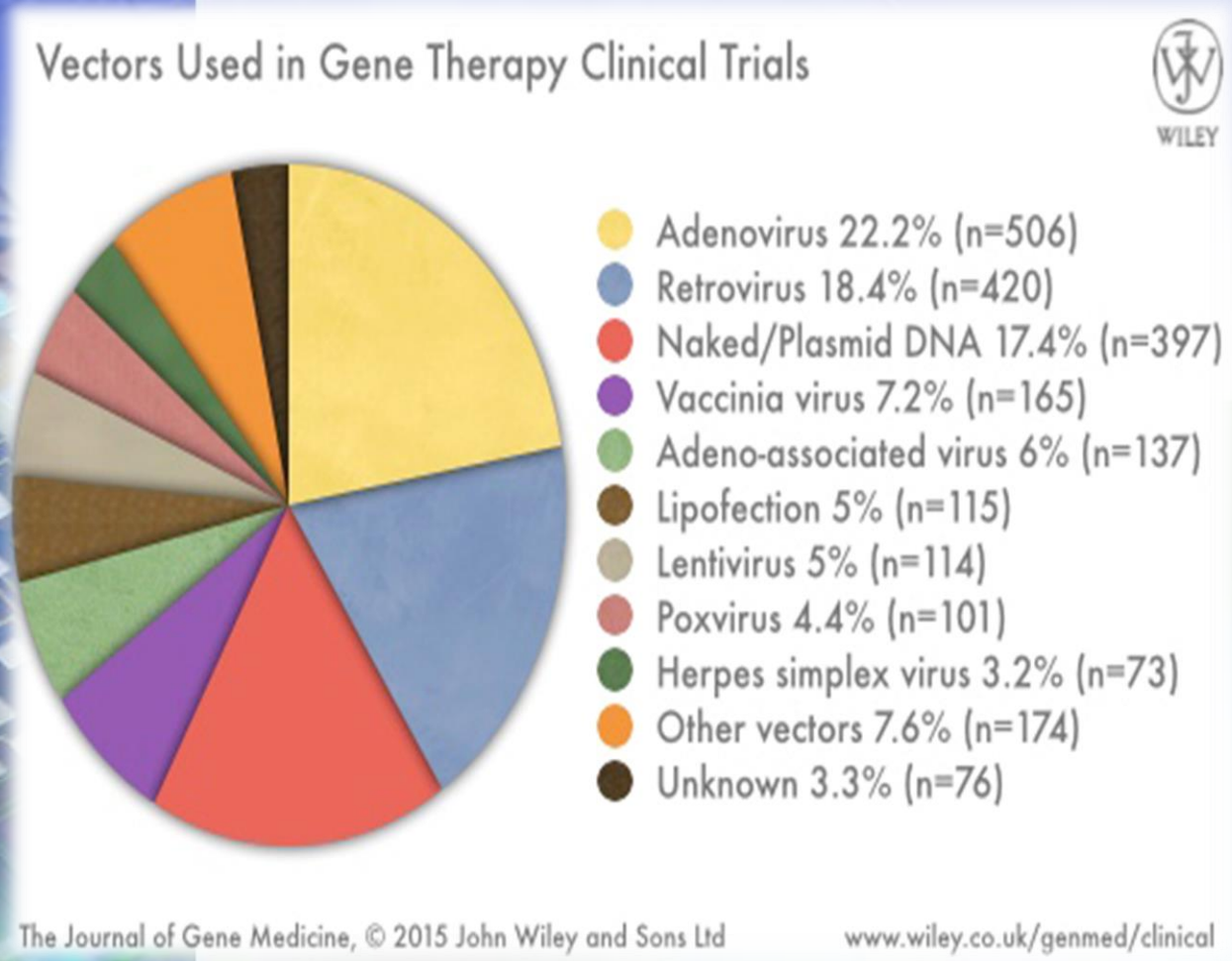
- Non-viral Vectors
  - non-toxic
  - no immune response
  - Lower tissue specificity
  - Less efficient gen transfer than viruses

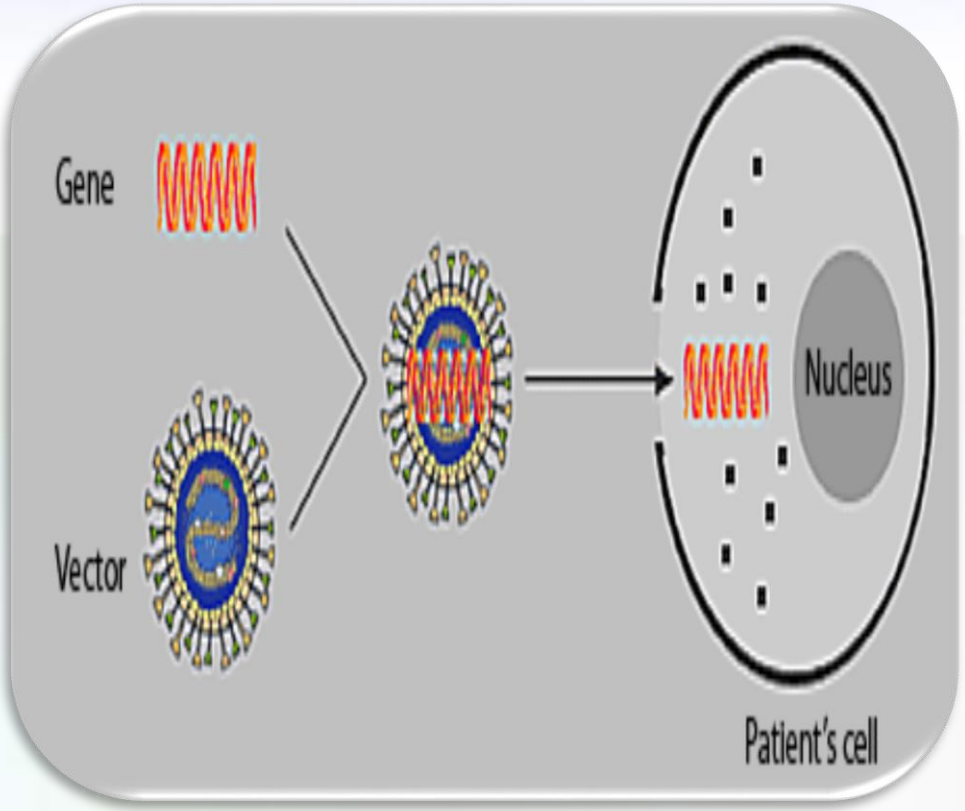


Types of vector



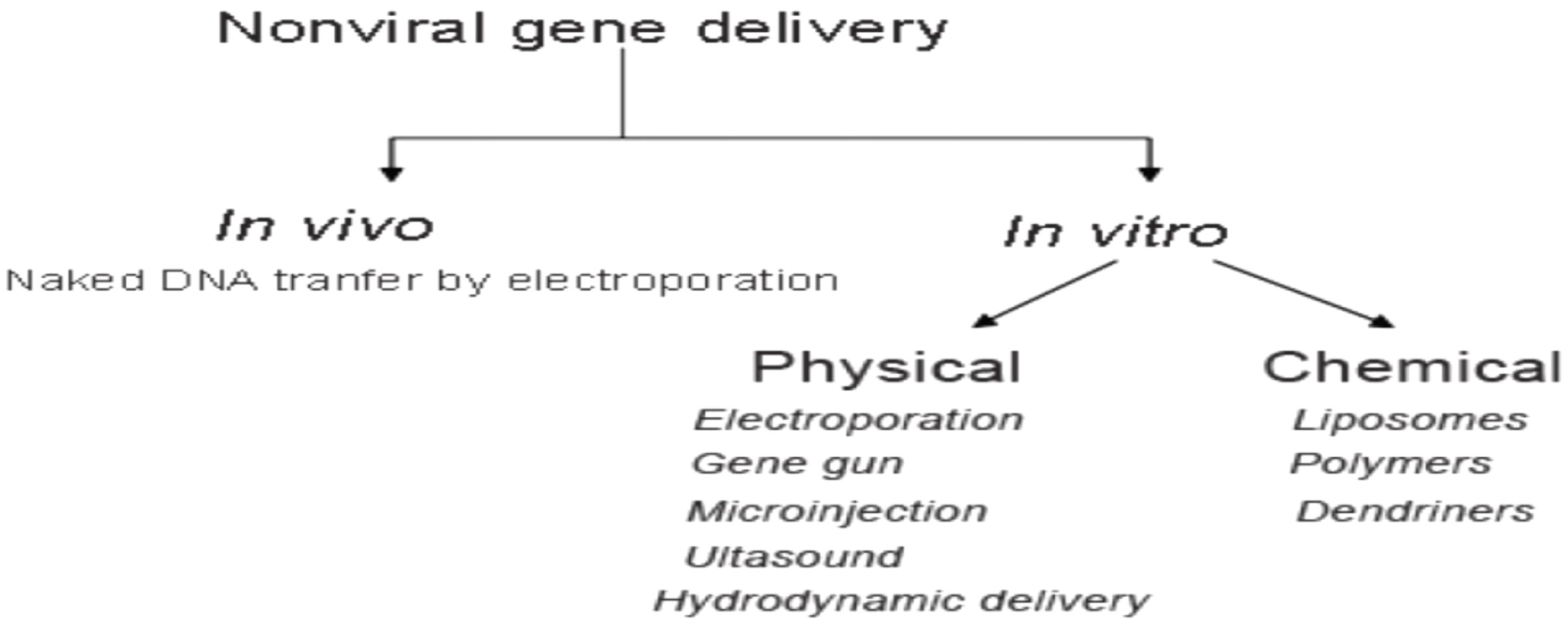
Types of vector





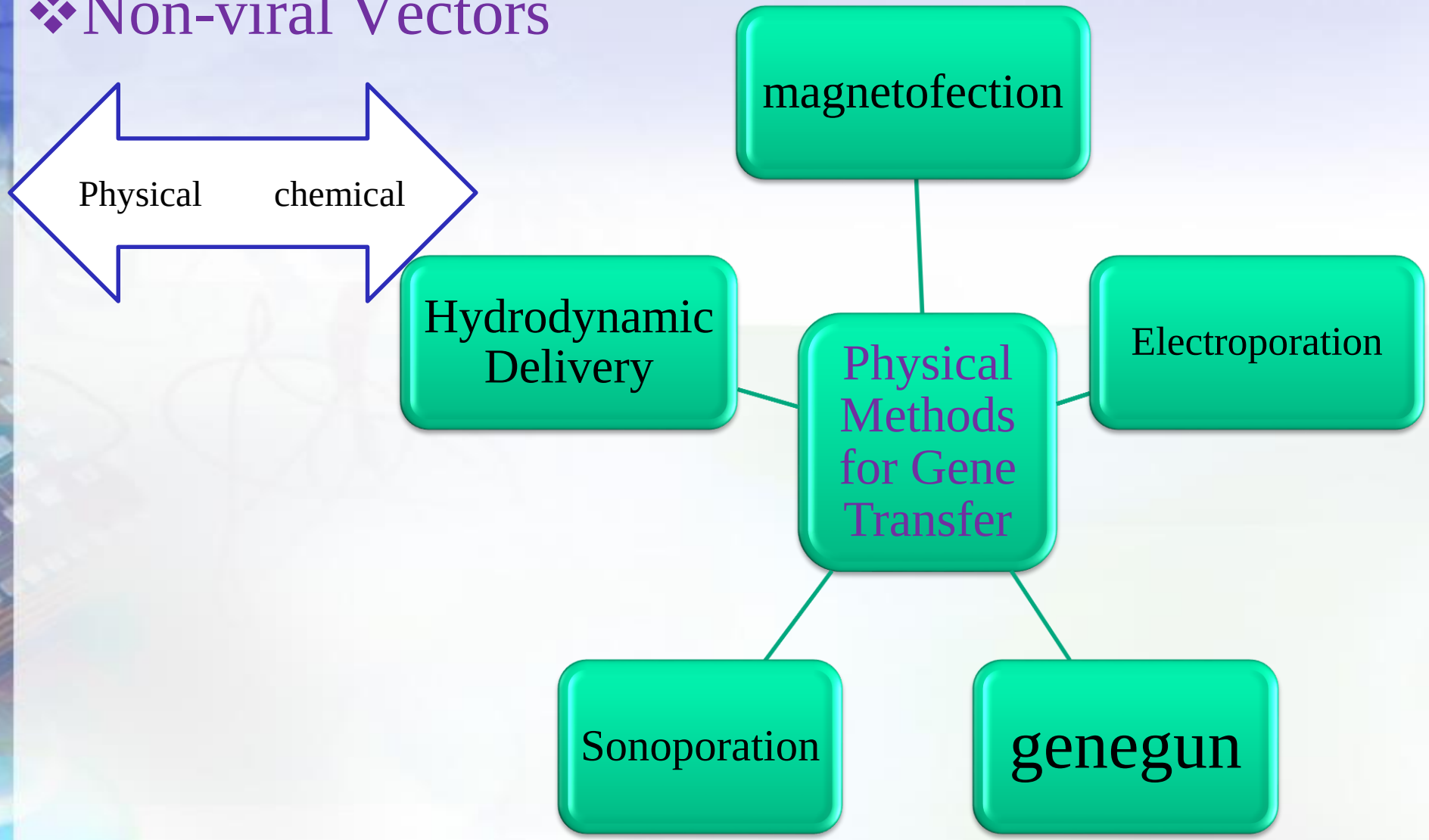
Types of vector

❖ Non-viral Vectors



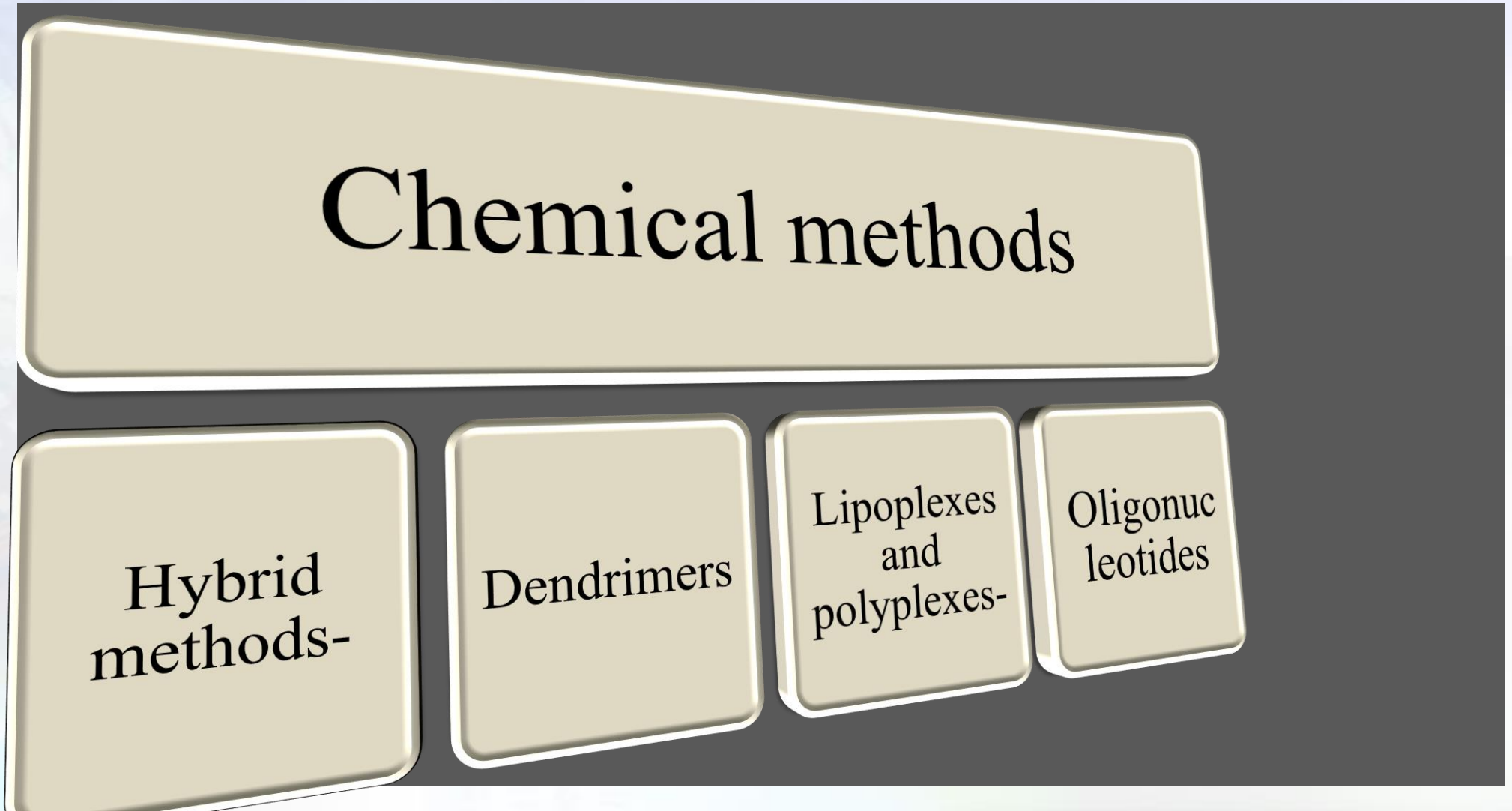
Types of vector

❖Non-viral Vectors

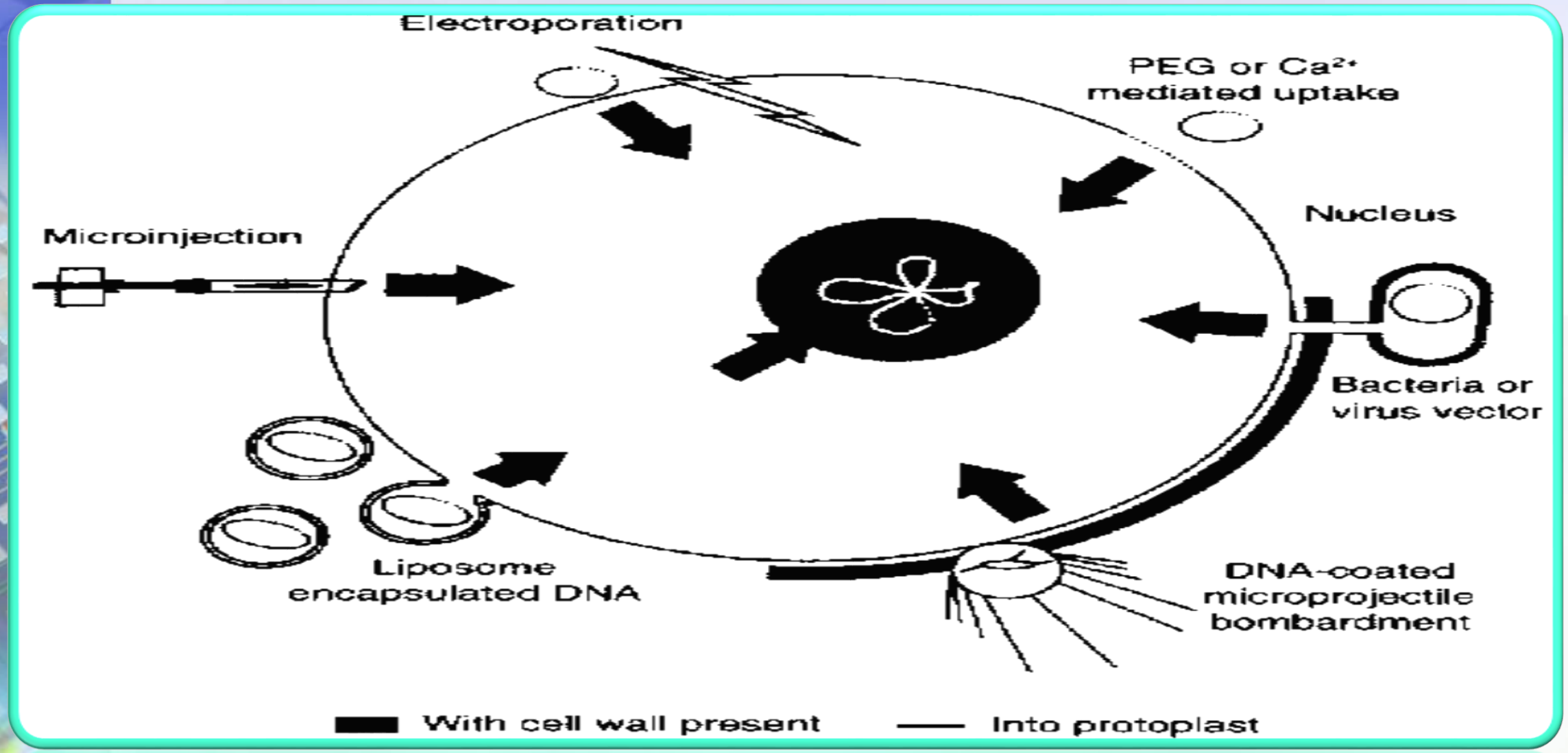


Types of vector

- Non-viral vectors



Types of vector



Advantage and disadvantages of vectors ⇨

Biologic viral vectors

Type of vectors	Integration To genome	Advantages	Disadvantages
Retrovirus	Yes	-Wide host range. -high **efficiency**. -transduction of **dividing cells**. -**stable integration**. - infects only once and does not replicate in vivo.	-Does not infect **non dividing** -insert size **5-7** kb.
Lentivirus (HIV)	Yes	-Wide host range. -stable transduction of **dividing and non dividing** -long term expression. -**nonpathogenic**. -lack of expression of **viral proteins**.	

Type of vectors	Integration To genome	Advantages	Disadvantages
Adenovirus	No	- Transduction of **non dividing** cells with high efficiency. -wide host rang. -high viral titer and high expression levels. -newly developed gutless vectors have insert size as large as **30** kb.	-Expression of viral proteins results in toxic reaction and inflammation. -**carcinogenic**. -low efficiency in dividing cells. -short-term expression. - insert size only **7-11** kb.
Adeno-associated virus	Yes	-Transduction of **dividing and non dividing** cells. -**non immunogenic** and **nonpathogenic**. -long-term expression of transgene.	-**Limited** transduction efficiency. -low efficiency of integration to genome. -small insert size~**4-5** kb.

Type of vectors	Integration To genome	Advantages	Disadvantages
Herpes simplex virus	No	-Transduction of **neurons and glial** cells. -wide host range. -large insert size up to **30** kb. -efficient infection.	-**Short-term** expression. -spreading of the infection to surrounding cell populations. - -terminally differentiated cells. -immunogenic

Advantage and disadvantages of vectors ⇨ Non viral vectors

Vector system	Advantages	Disadvantages
1-Nacked plasmid DNA	-simple, relatively efficient. -non immunogenic. -no mutagenesis.	-Transient gene expression. -DNA **not integrated** into the genome. - remain episomal.
2- Chemical vectors *a-Calcium phosphate* *b-Cationic liposomes (lipoplex)* *c-Polylysine-DNA complexes*	- **Easy** to use. -Non infectious. -non **immunogenic**. -effective for in vivo gene transfer. -can carry large DNA fragments.	-Random integration. -inefficient DNA transfer. -Unstable. -remain episomal. -poor gene expression.

Non viral vectors

Vector system	Advantages	Disadvantages
3- Physical methods *a- Electroporation* *b-Gene gun* *c-Microneedle injection*	-Easy to use. -**safe.** -Cell receptor independent. -delivers genes to different tissues. -used in vaccine protocols. -increase gene expression.	-**Random** integration. -inefficient DNA transfer. -**No integration** of DNA. - transient gene expression. - Ineffective in large surface area.
4-Biologic non viral vectors *(human artificial chromosomes)*	-**Stable**. -non-infectious. -can carry large fragments of DNA. -non immunogenic. -no integration into the genome.	- Still in developmental stages.

Advantage and disadvantages of vectors

❖ *Integrating versus Non-Integrating Viruses(Viral Vectors)*

✓ ***Integrating viruses***

- Retrovirus (e.g. murine leukemia virus)
- Adeno-associated virus (only 4kbp accommodated)
- Lentivirus

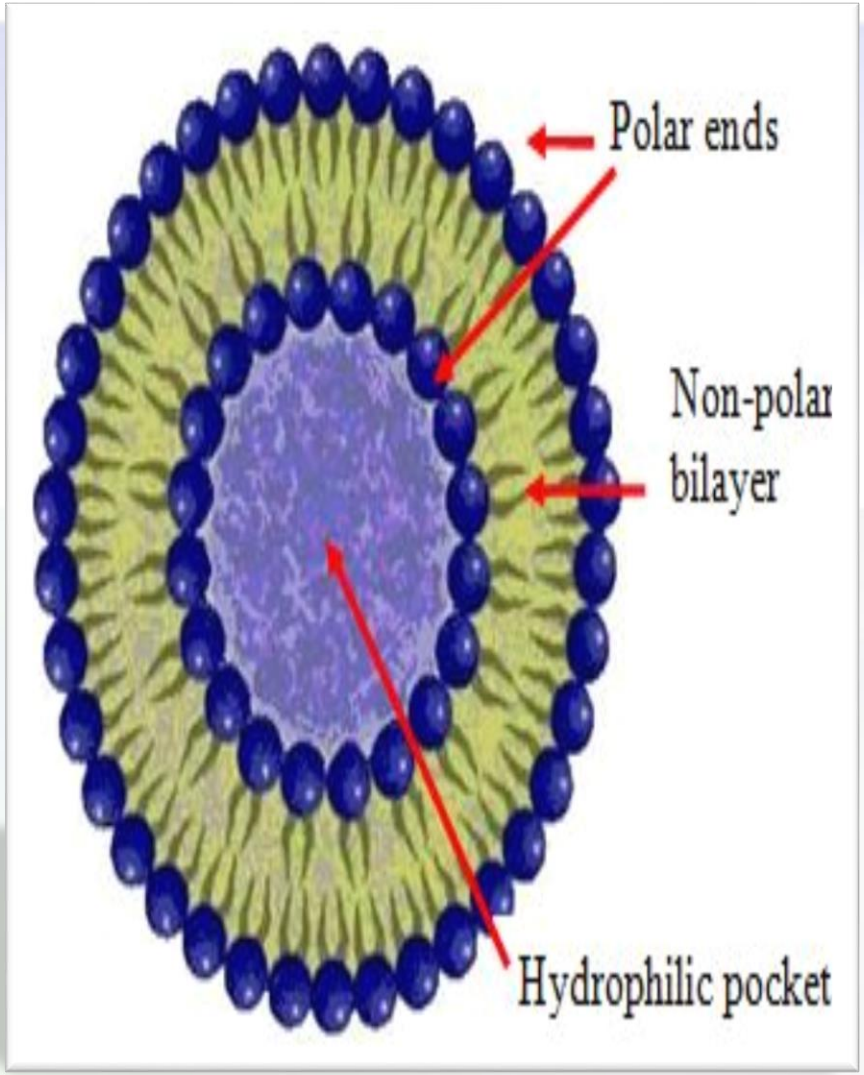
✓ ***Non-Integrating viruses***

- Adenovirus
- Alphavirus
- Herpes Simplex Virus
- Vaccinia

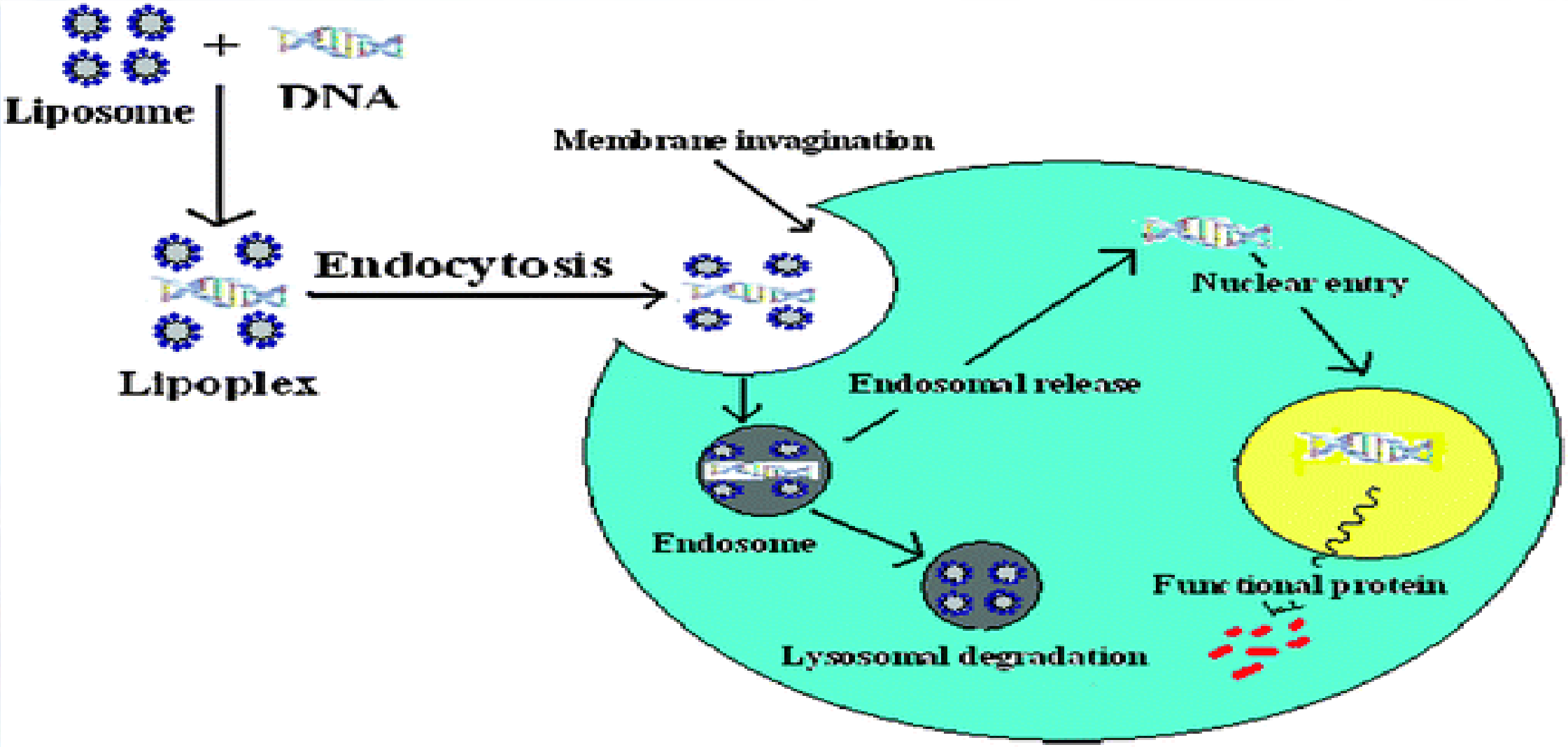
Non-viral vectors

Liposomes (17)

- ✓ not limited by size or number of genes
- ✓ safe
- ✓ easy to produce
- ✓ short-term expression
- ❑ Diverse manners of ‘lysing’ the liposome
- ✓ Temperature sensitive
- ✓ Target sensitive
- ✓ pH sensitive
- ✓ Electric field sensitive

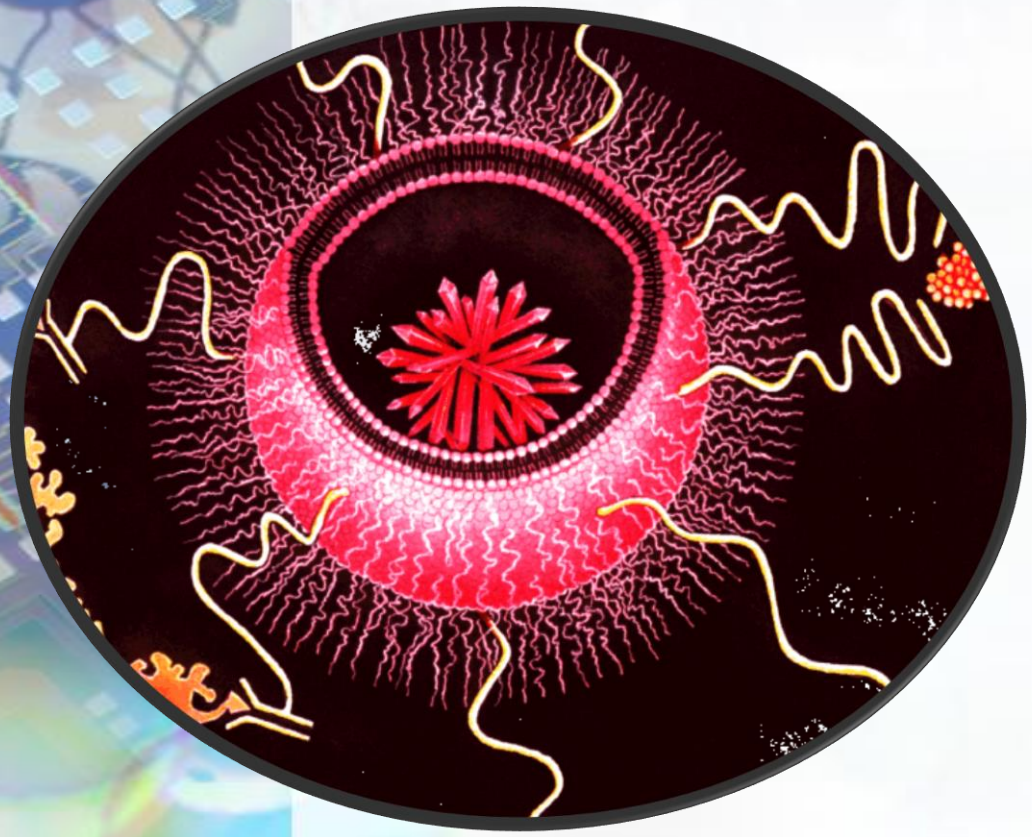


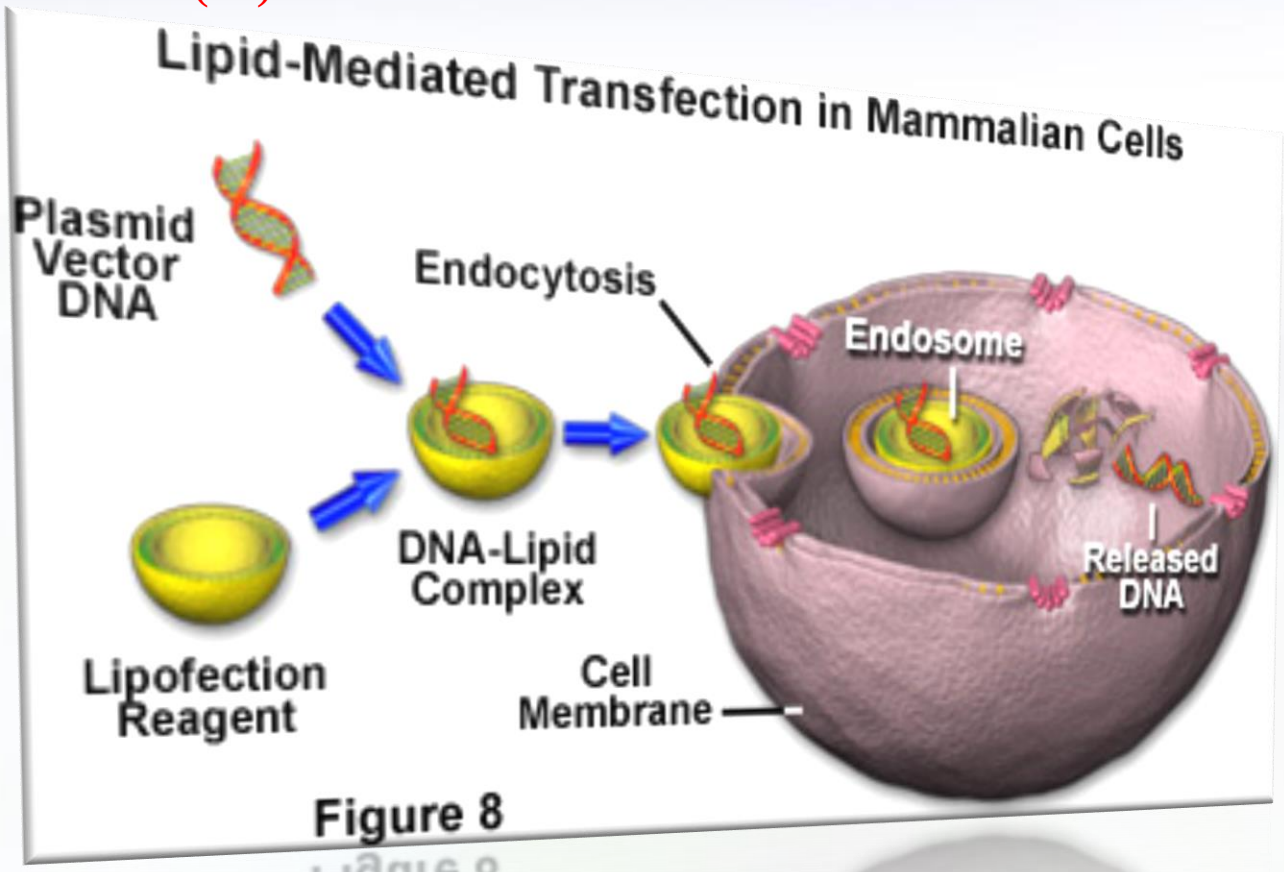
Liposomes


Liposome
+
DNA
Lipoplex
Endocytosis
Membrane invagination
Endosomal release
Nuclear entry
Endosome
Lysosomal degradation
Functional protein

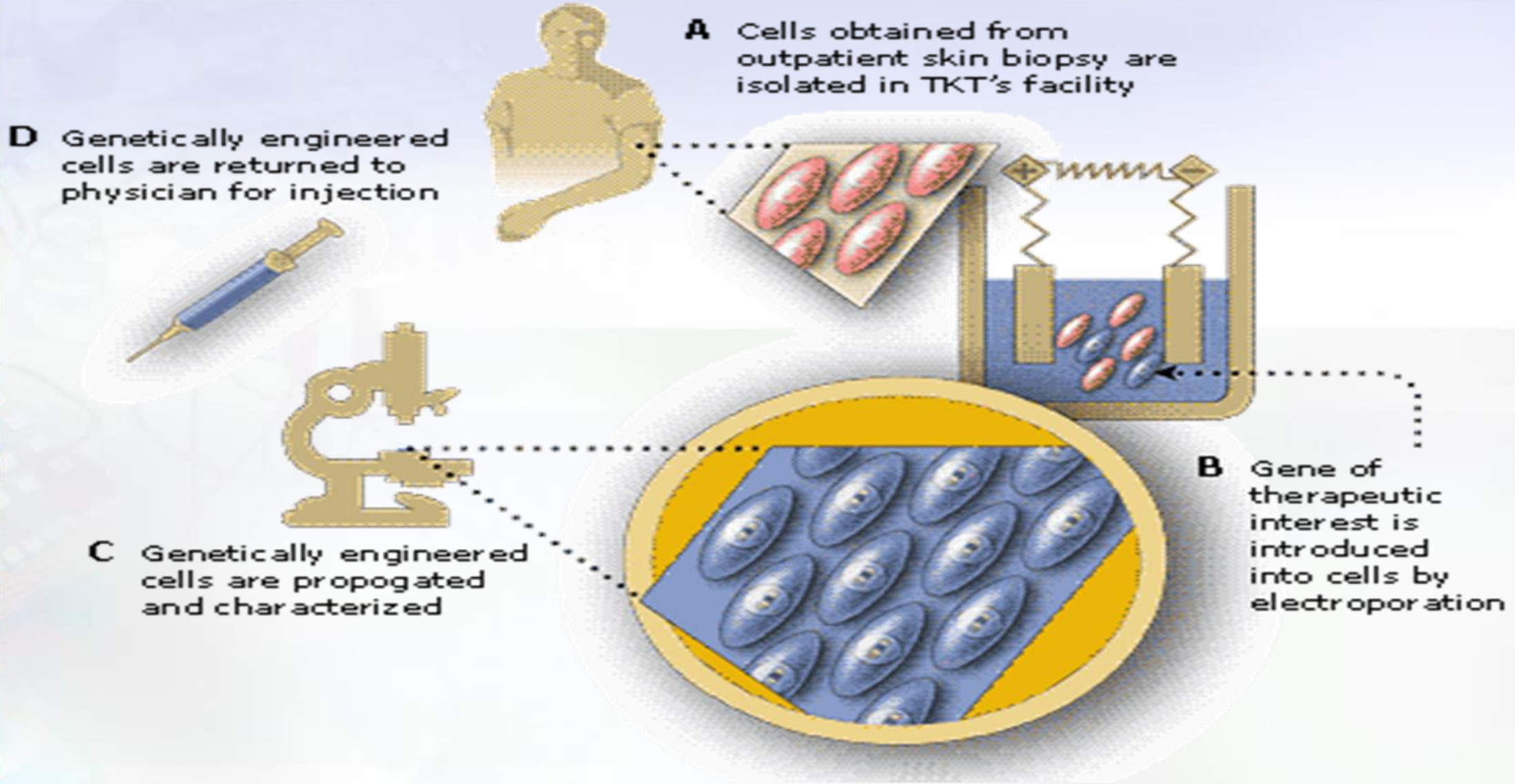
Liposomes

- Especially good for in-lung delivery (**cystic fibrosis**).(18)
- 100-1000 times more plasmid DNA needed for the same transfer efficiency as for viral vector.(18)



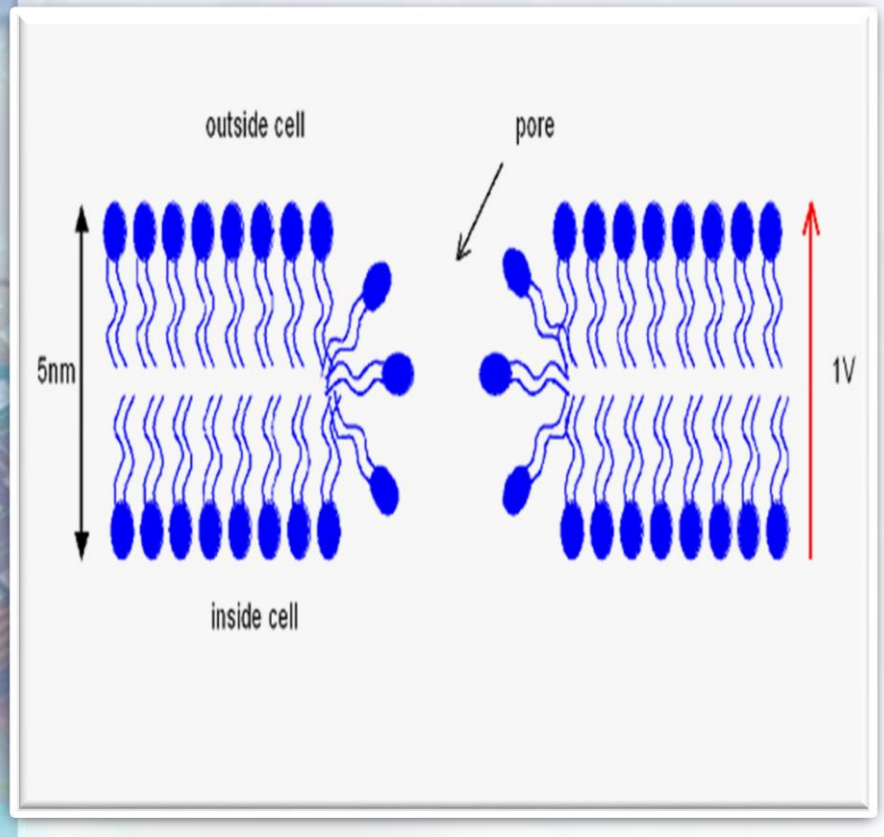


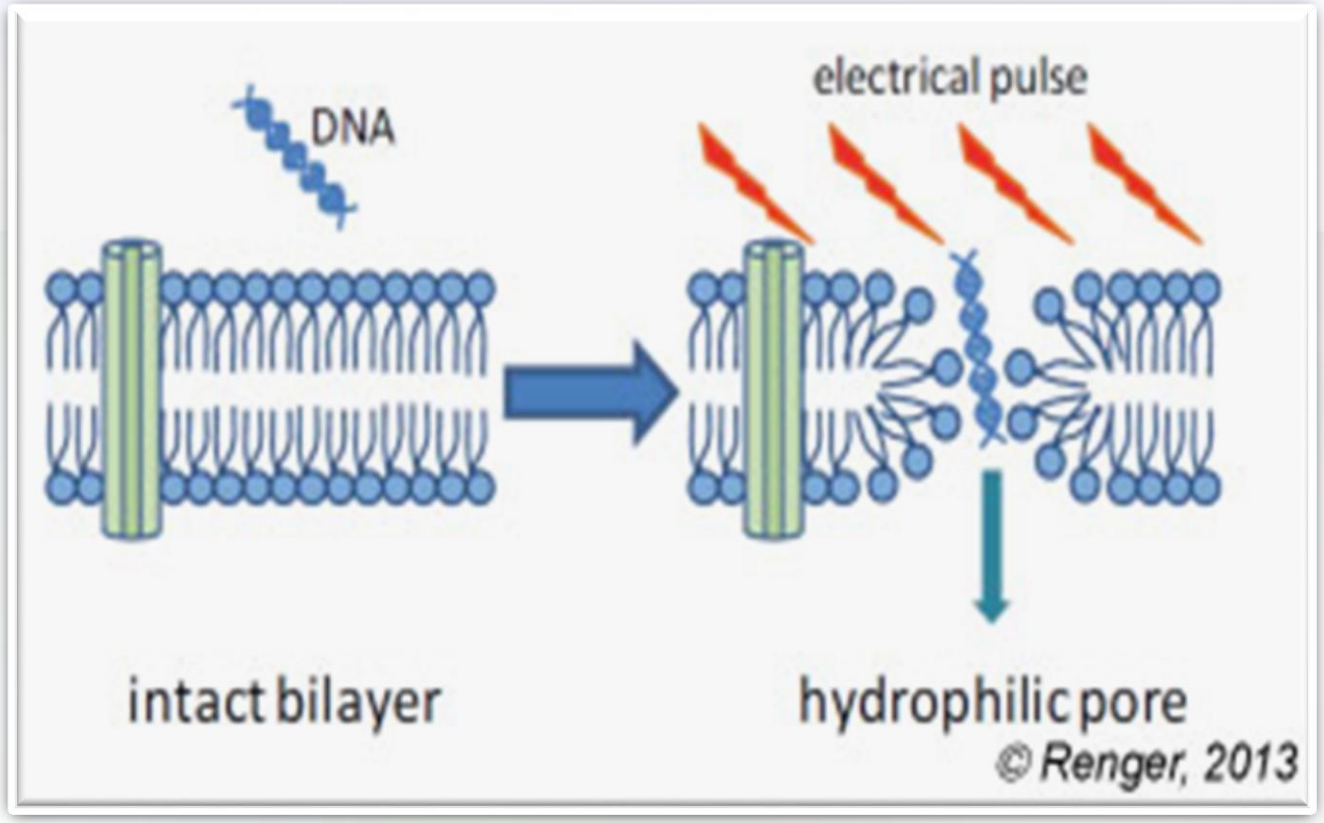
Electroporation



Electroporation

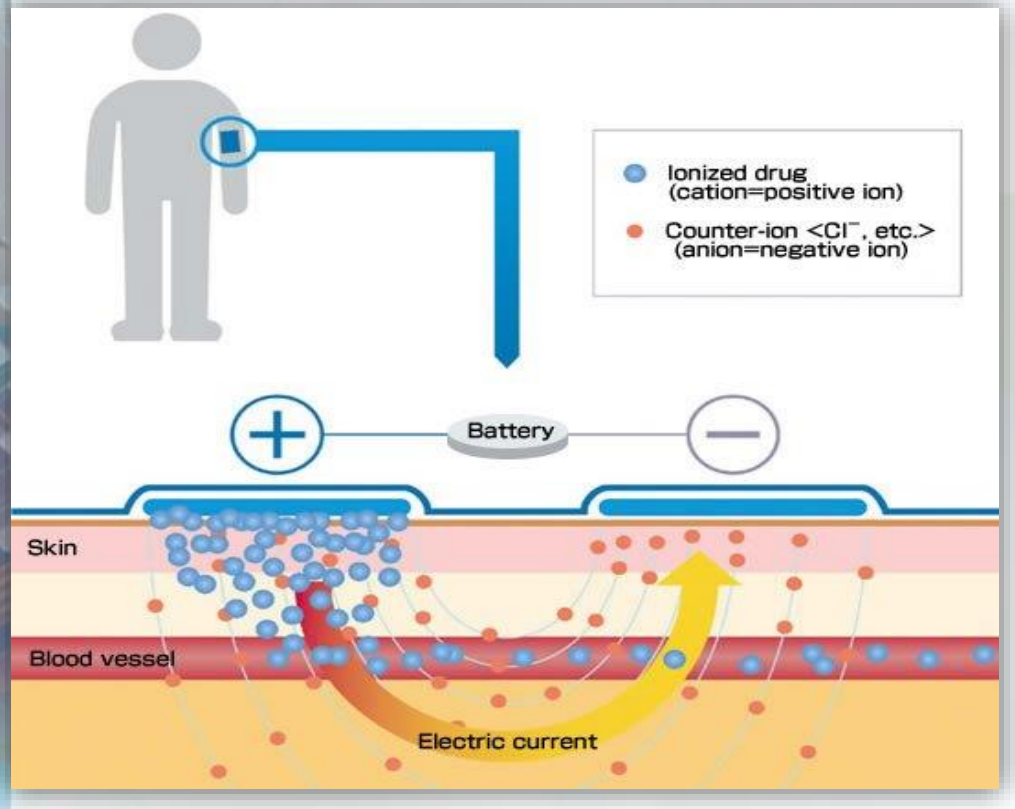
- ✓ Applying electrical pulses to cellular membranes to increase permeability.(19)
- ✓ facilitate the penetration of naked DNA molecules.(19)

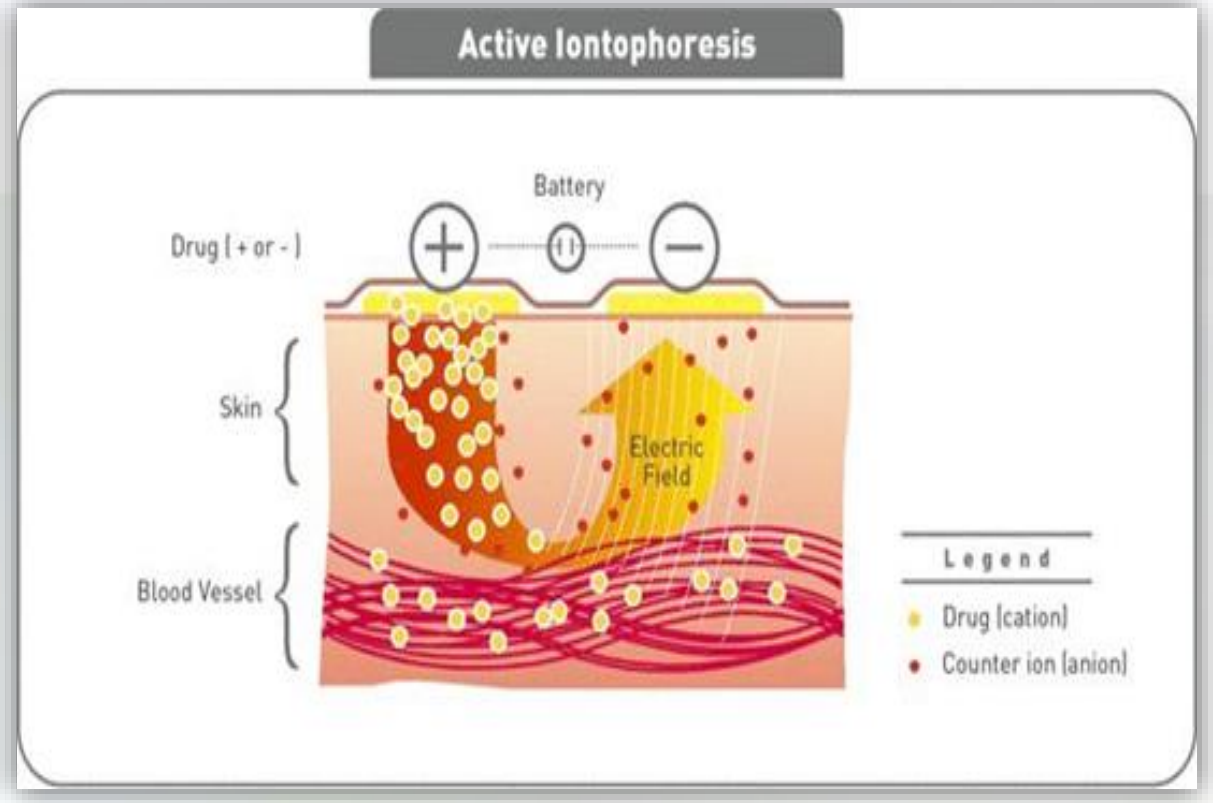




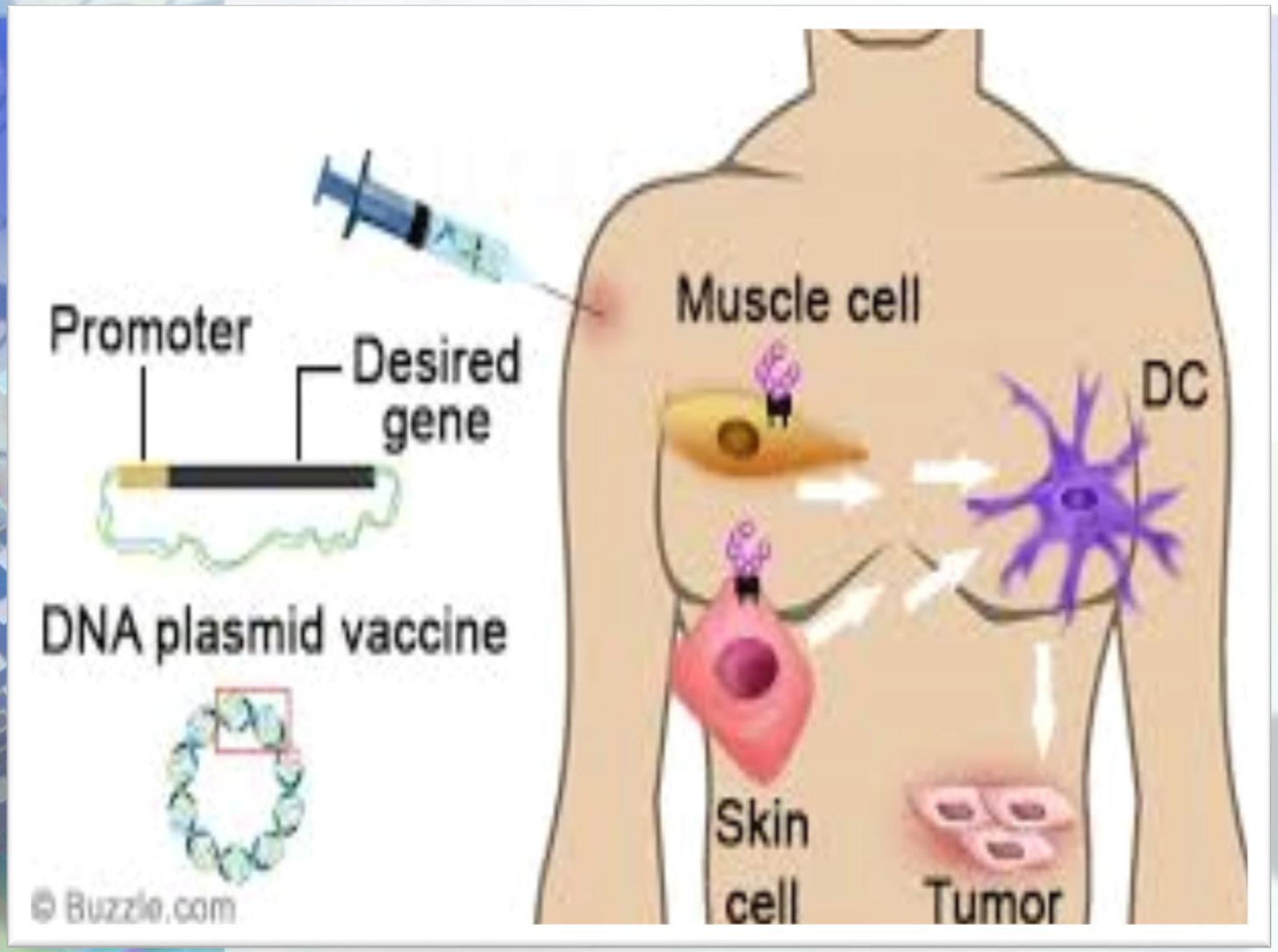
Iontophoresis

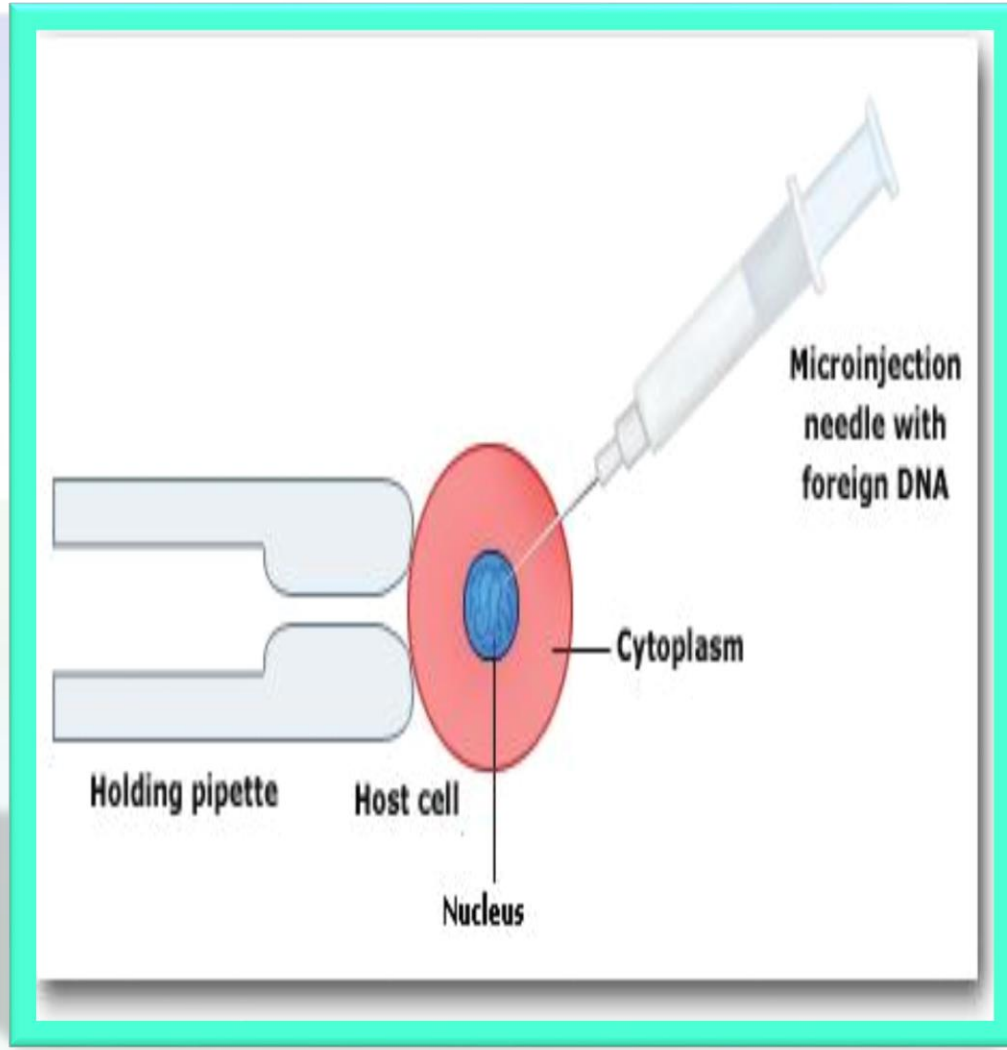
- ✓ charged molecules move according to an applied low voltage electrical current through a tissue within an electrical field.(19)
- ✓ Iontophoresis was coupled to intravitreal injection of oligonucleotides.(19)



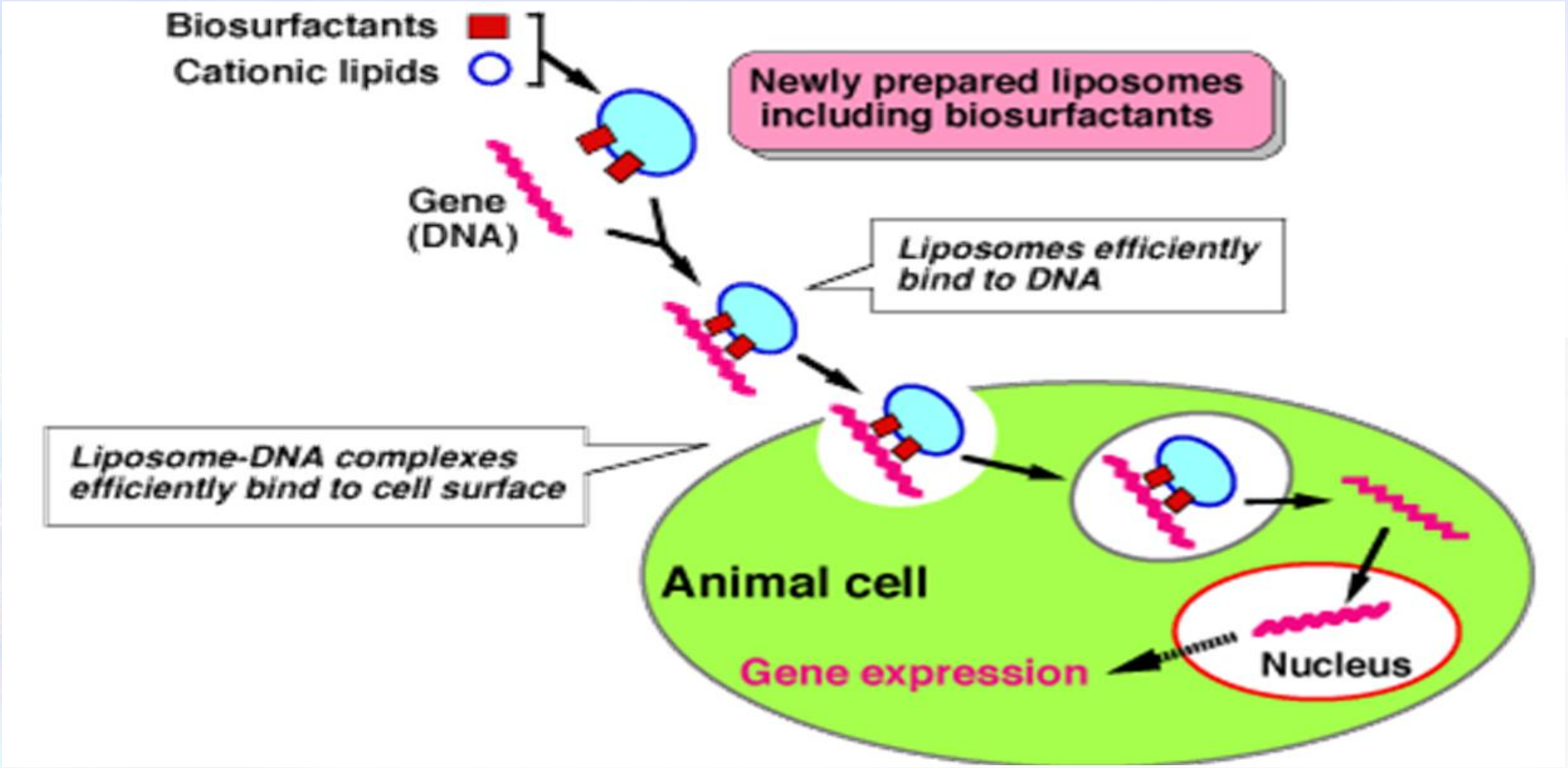


Injections of naked DNA



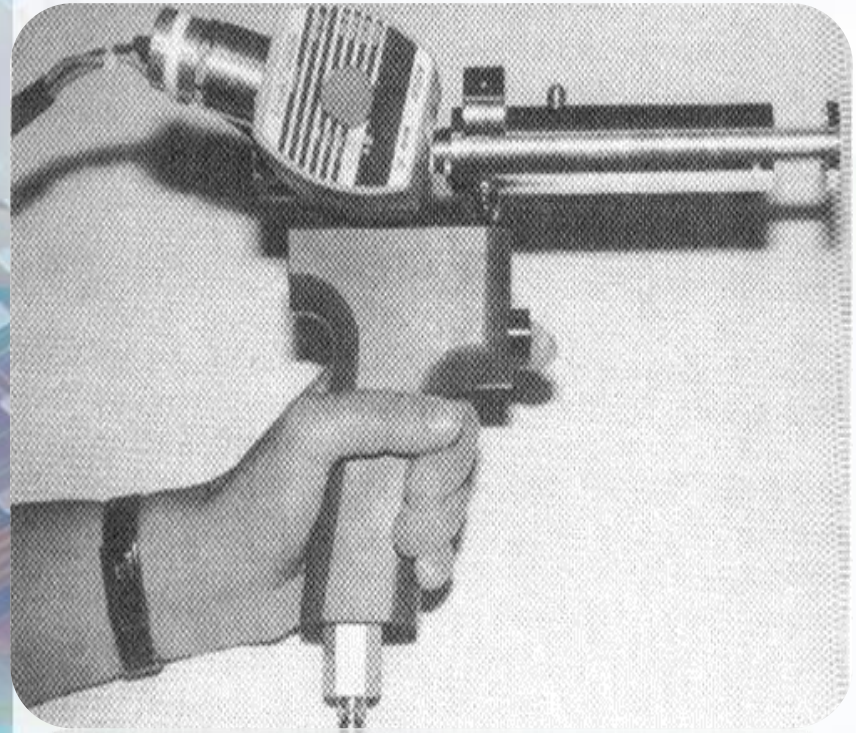


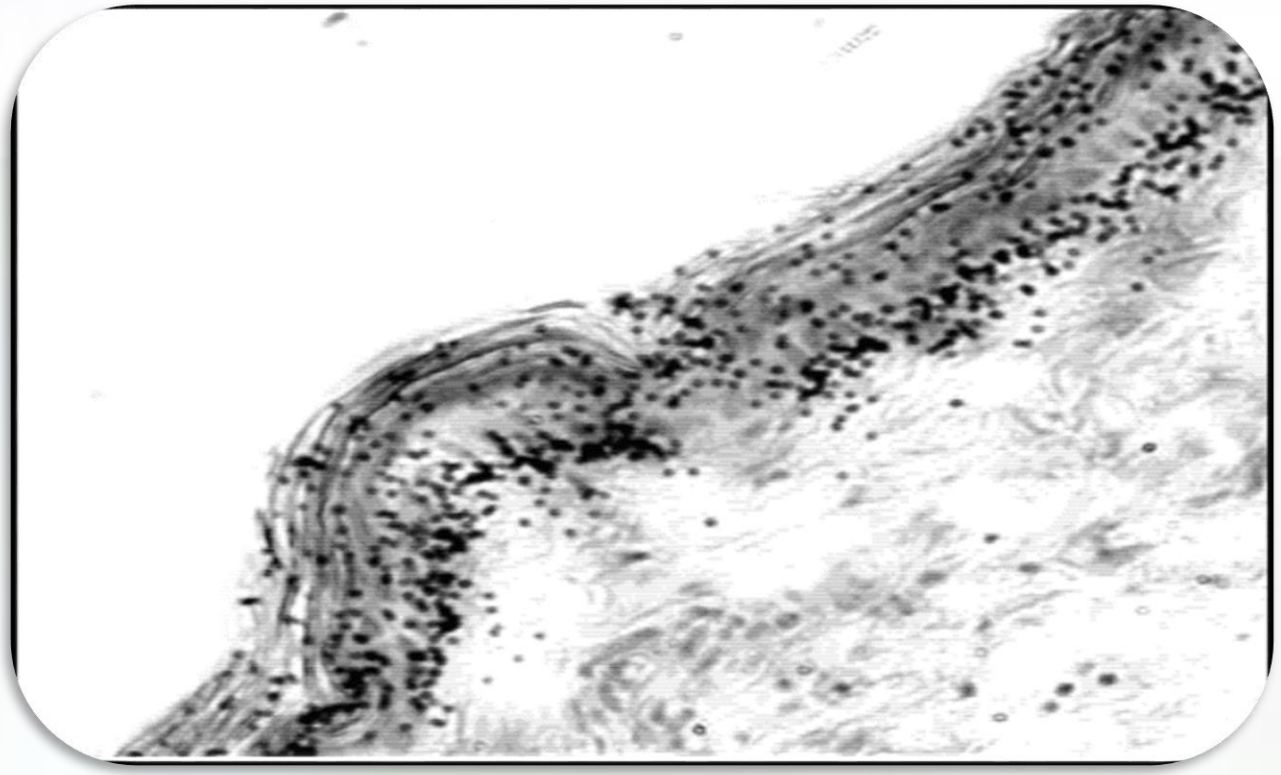
Amino acid polymers: cationic polymers



Ballistic DNA Injection (gene guns)

- Invented for DNA transfer to plant cells
- Fully applicable to eukaryotic cells (4)





Receptor-mediated endocytosis

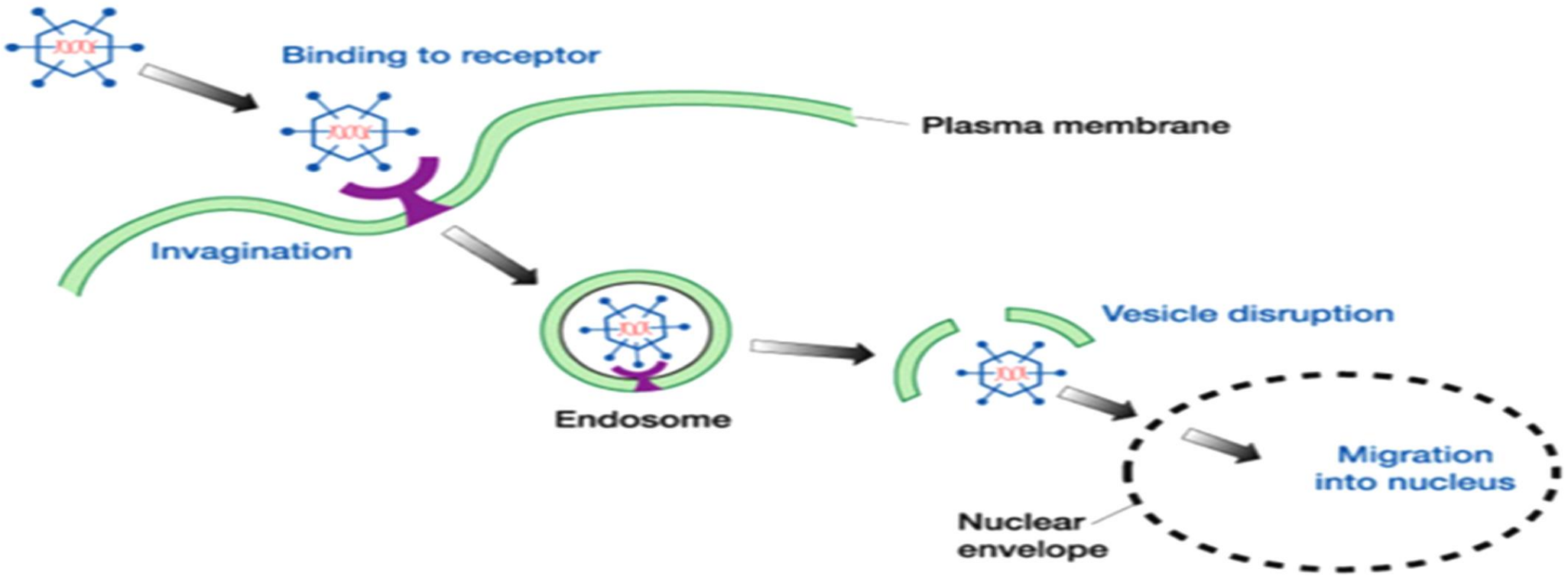
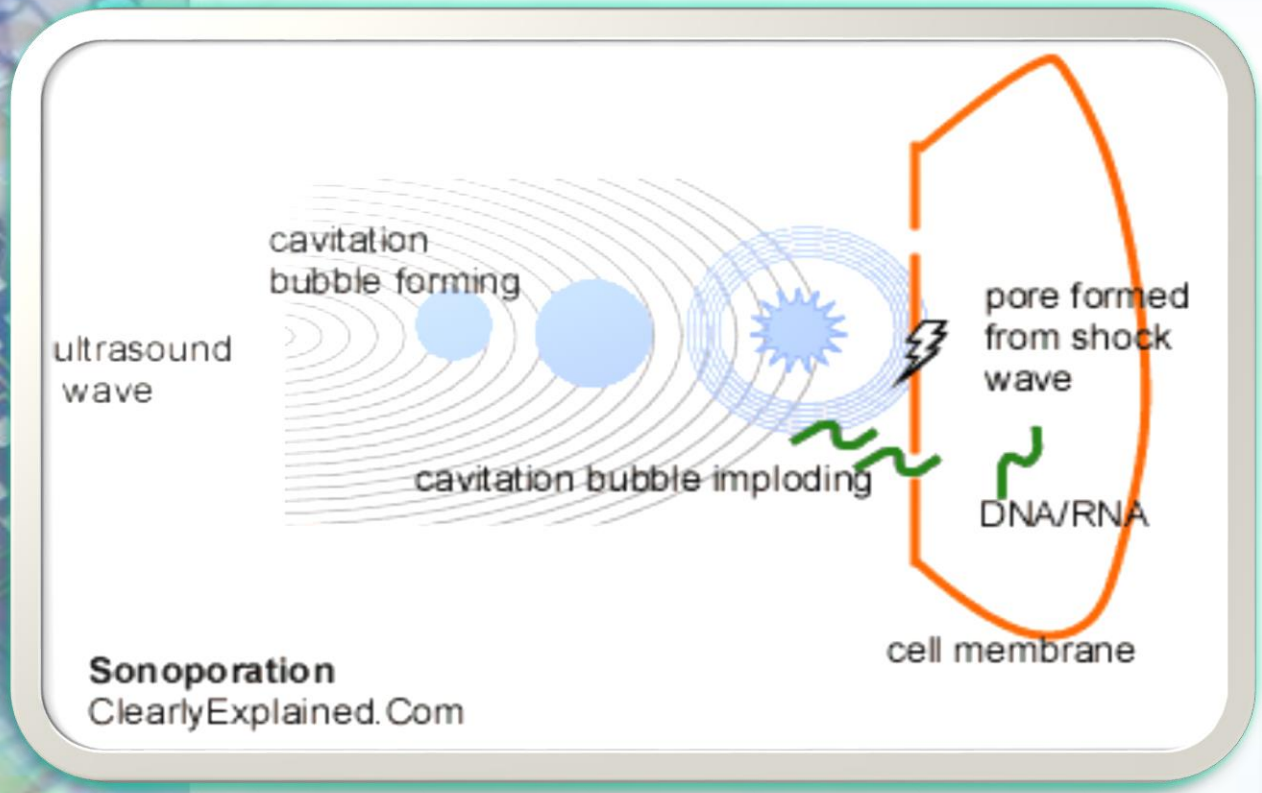
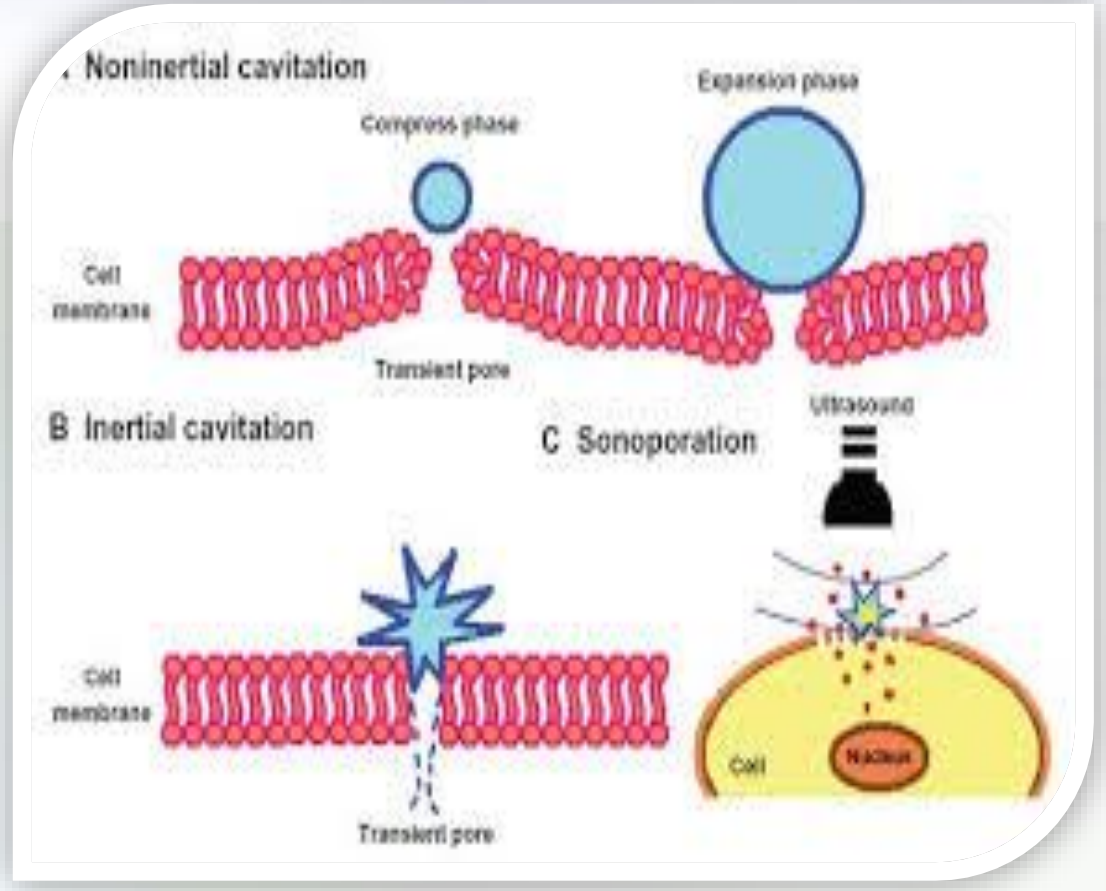


Figure 21-9 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Sonoporation

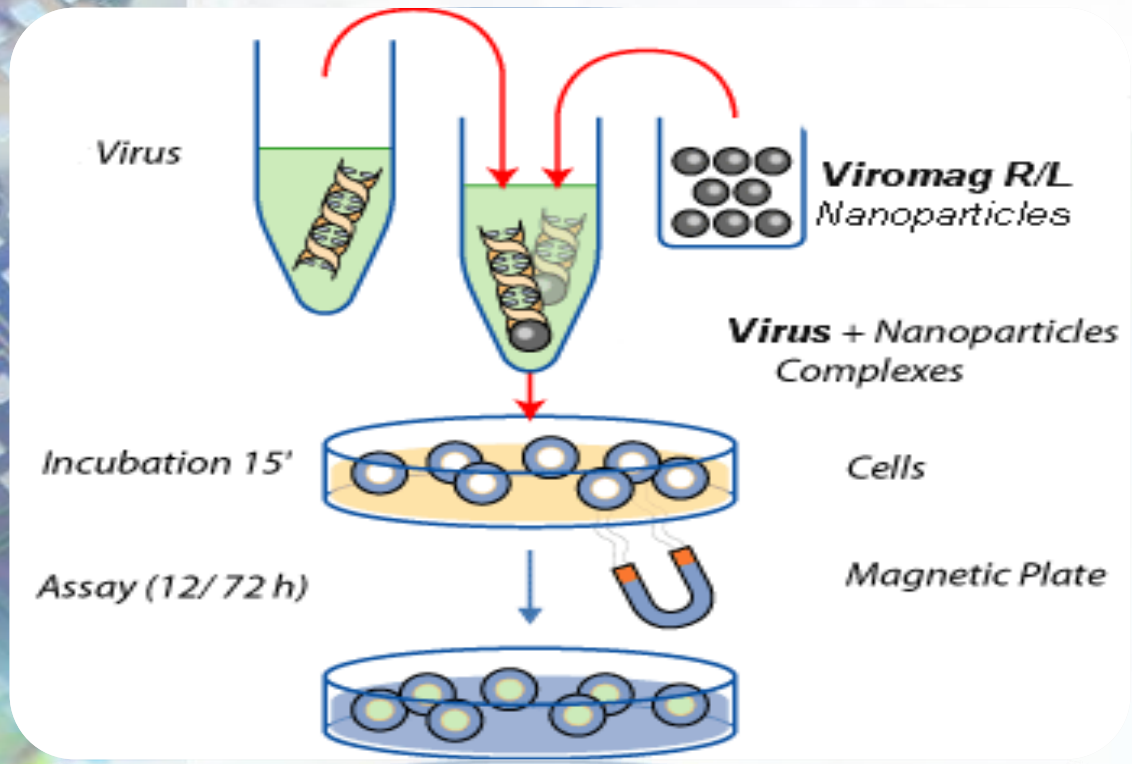
- ✓ Uses ultrasonic frequencies to deliver DNA(4)
- ✓ The process disrupt the cell membrane and allow DNA to move into cells.(4)

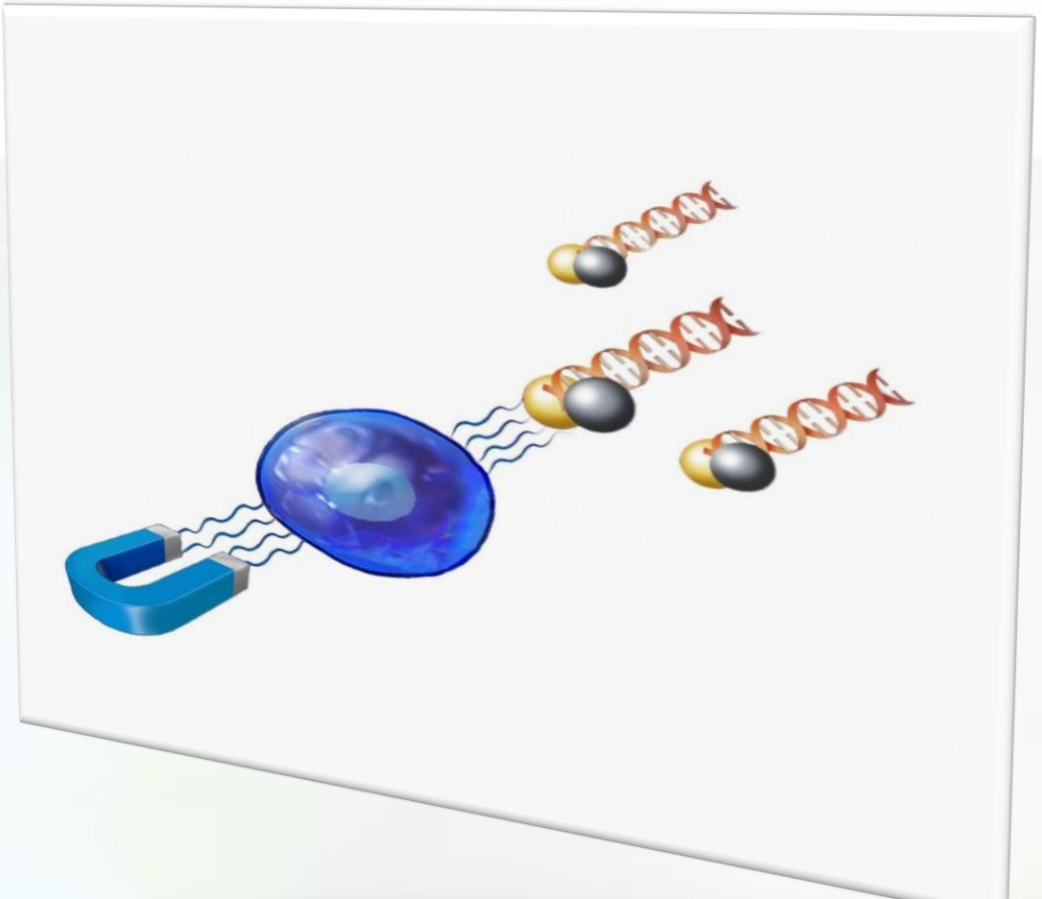




Magnetofection

- ✓ DNA is complexed to a magnetic particles (4)
- ✓ A magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer.(4)

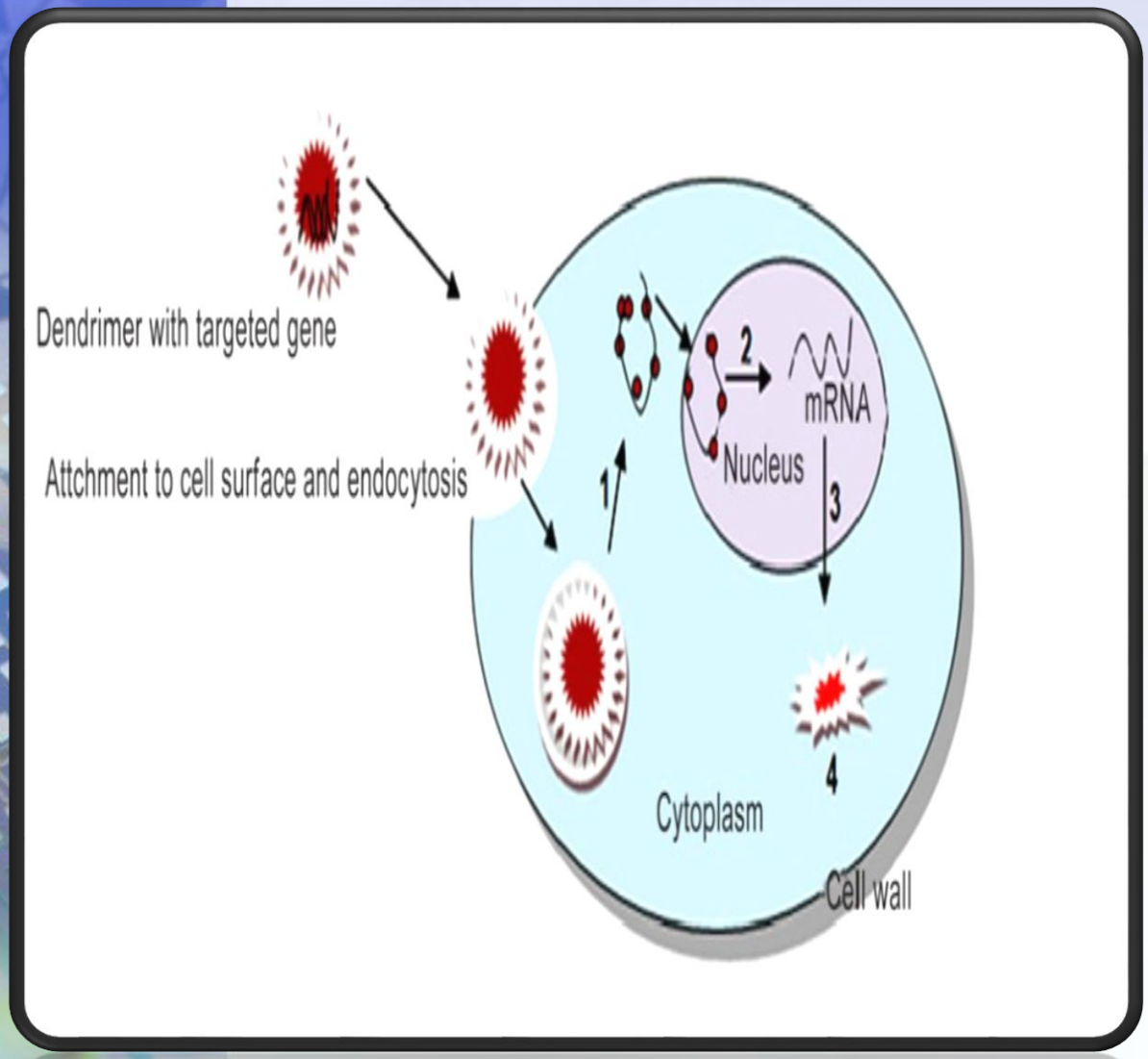


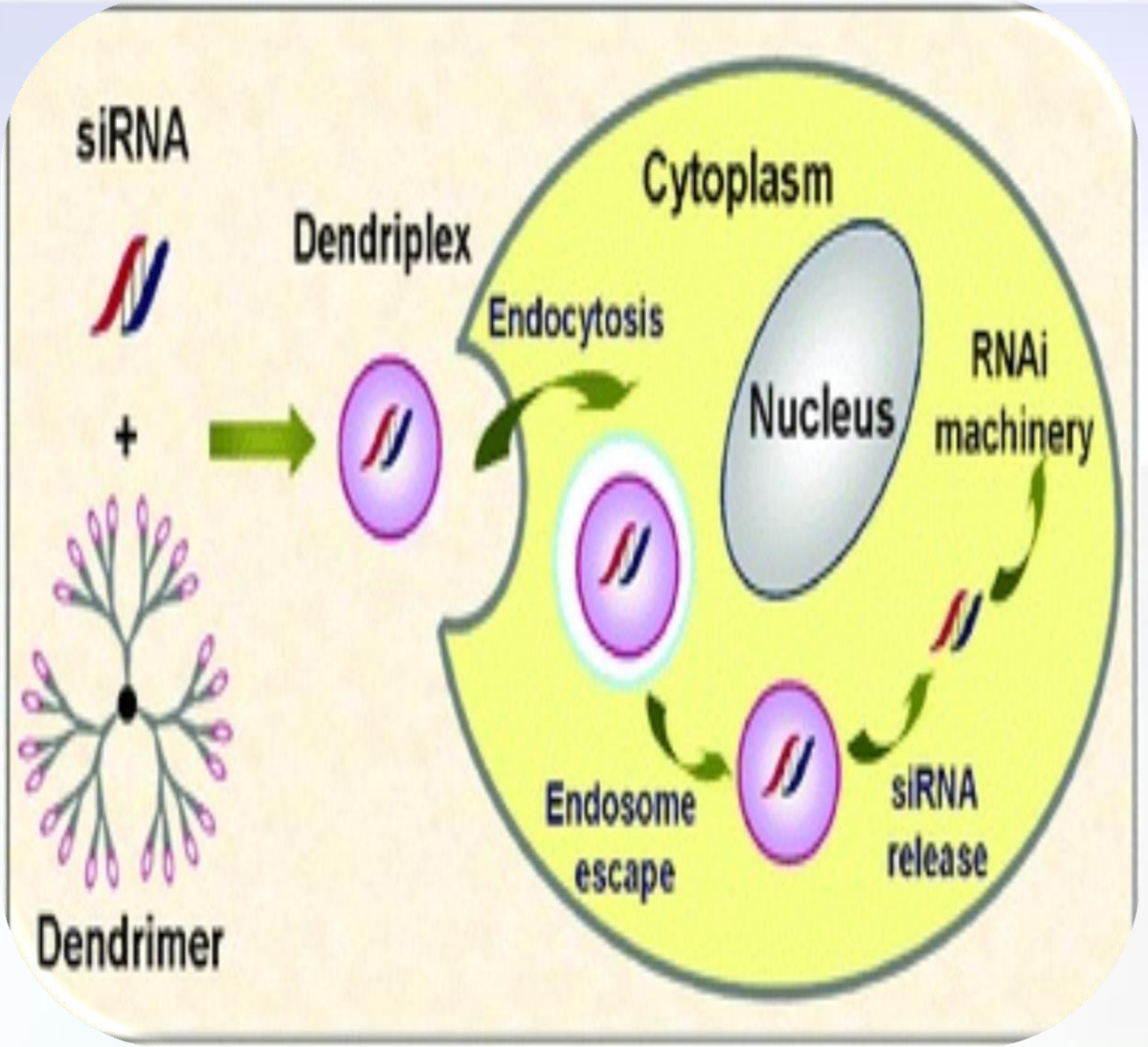


Dendrimers

- ✓ A highly branched macromolecule with a spherical shape.(17)
- ✓ The surface of the particle may be functionalized in many ways(17)
- ✓ In the presence of DNA or RNA charge complimentarity leads to a temporary association of the nucleic acid with the cationic dendrimer
- ✓ Taken into the cell via endocytosis.(4)

Dendrimers

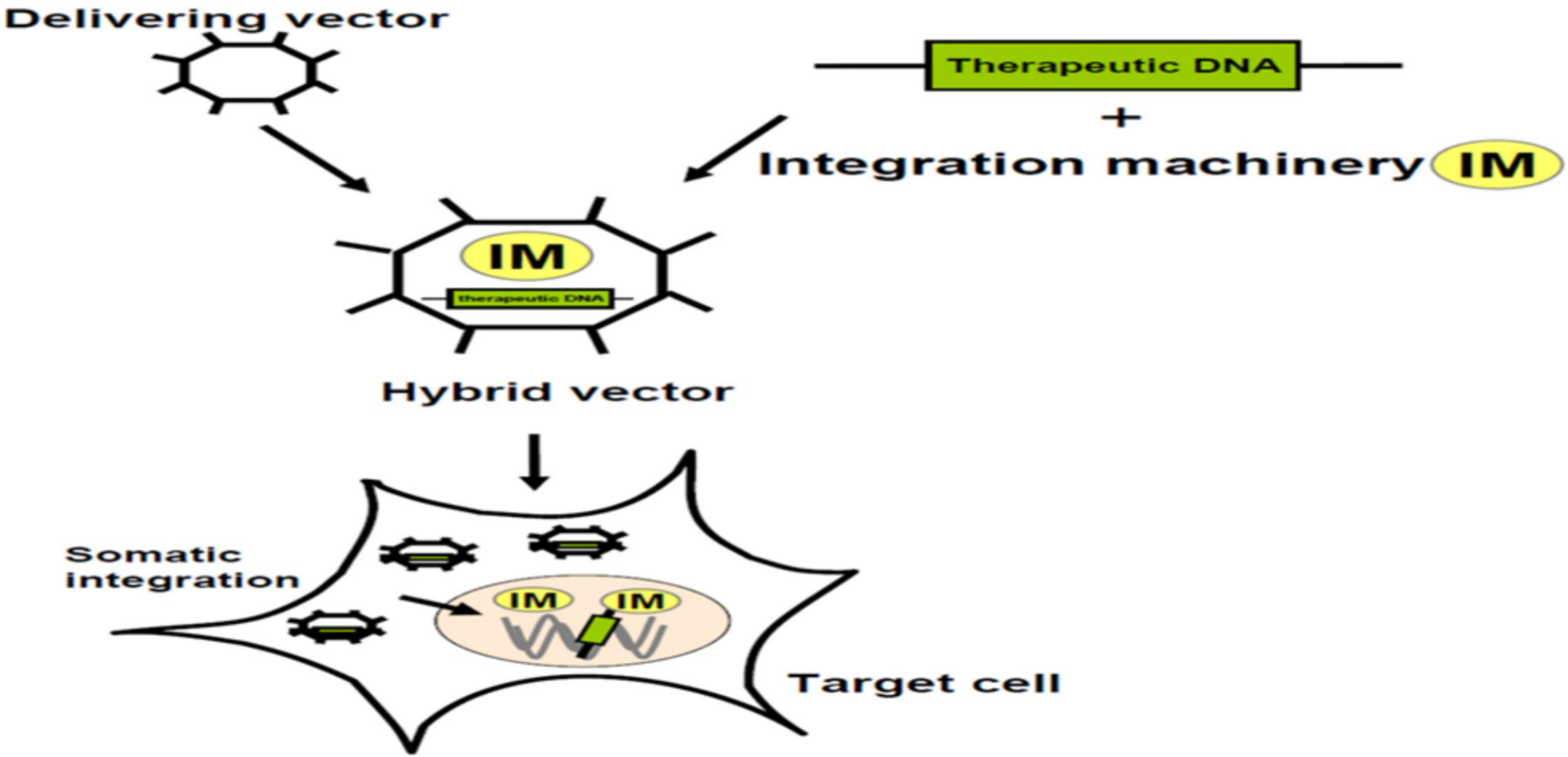

Dendrimer with targeted gene
Attchment to cell surface and endocytosis
1
2
mRNA
Nucleus
3
4
Cytoplasm
Cell wall


siRNA
+
Dendrimer
Dendriplex
Endocytosis
Cytoplasm
Nucleus
RNAi machinery
Endosome escape
siRNA release

Hybrid methods

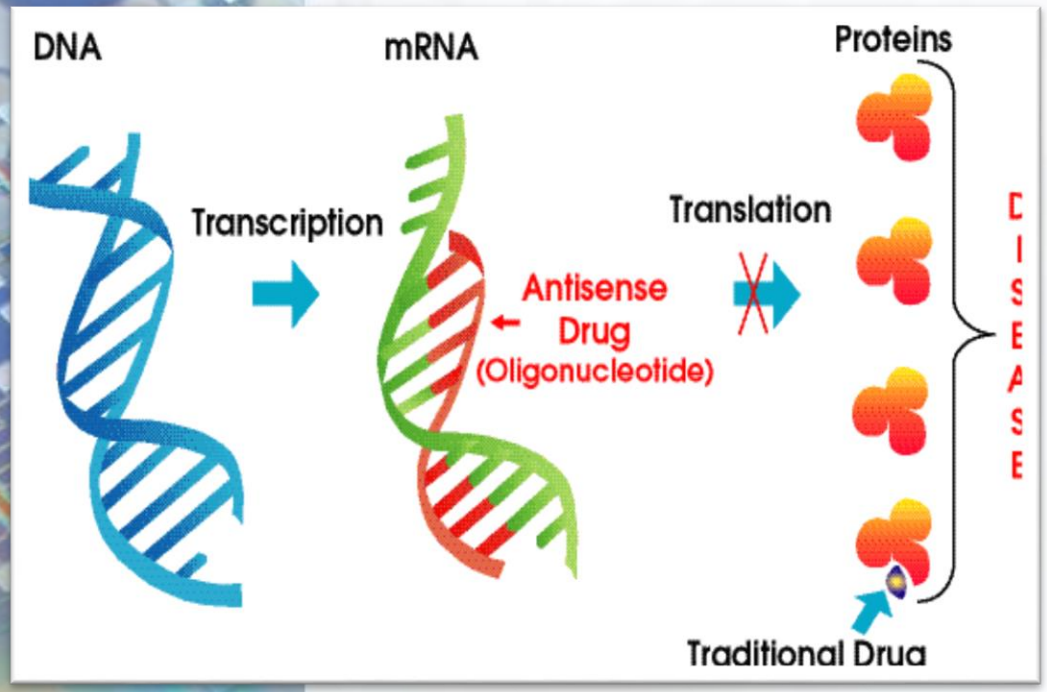
- ✓ Combine two or more techniques.(4)
- ✓ Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus.(4)
- ✓ More efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods(4)

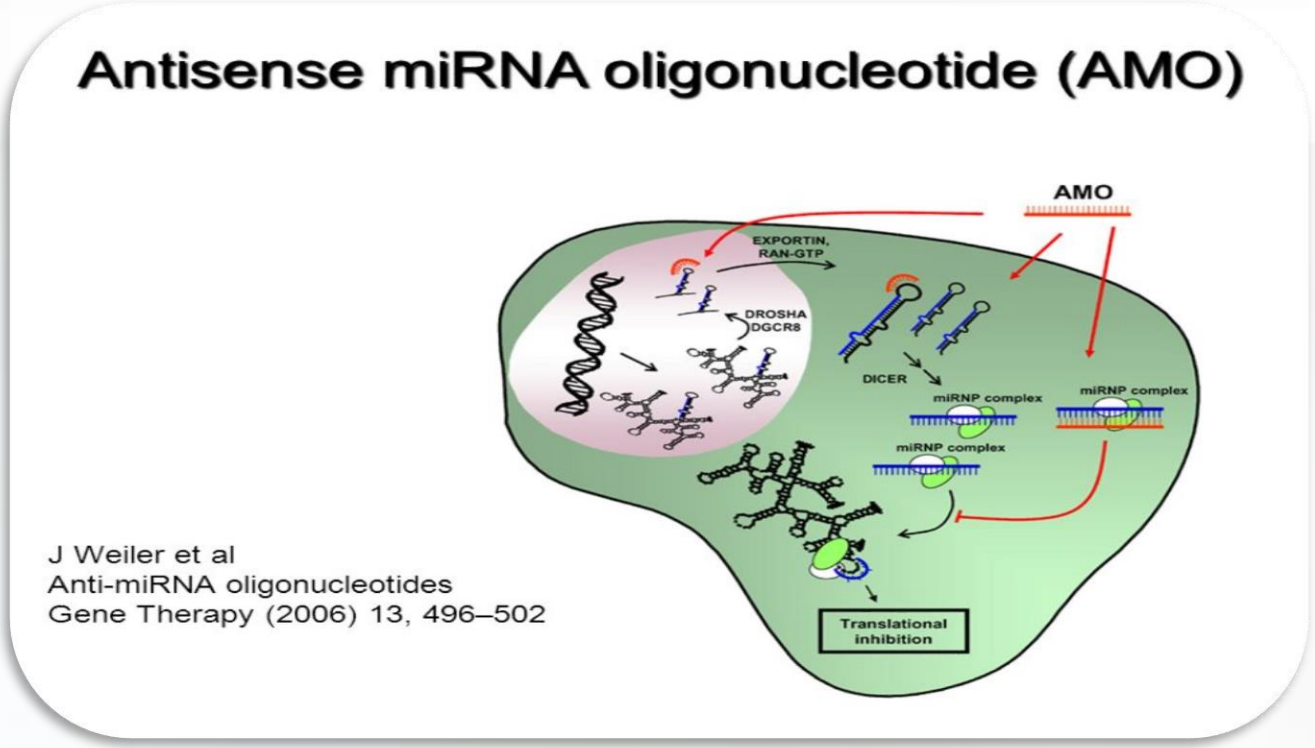
Hybrid methods



Oligonucleotides

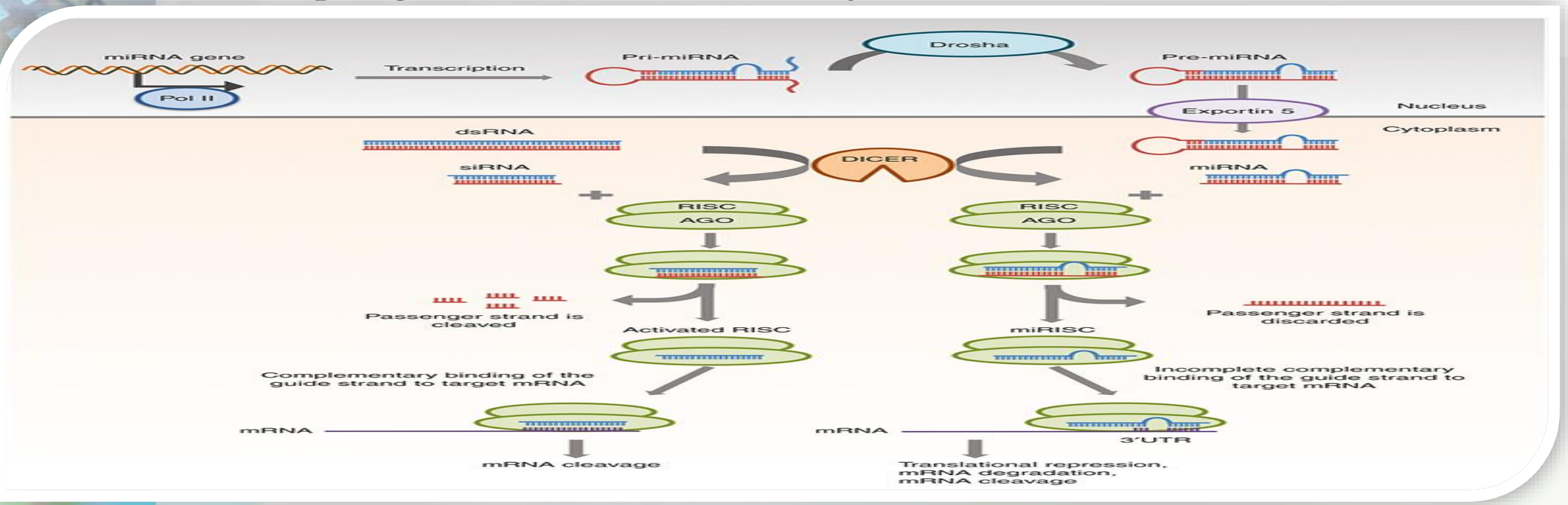
- ✓ Inactivate the genes involved in the disease process.(20)
- ✓ One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene.(20)





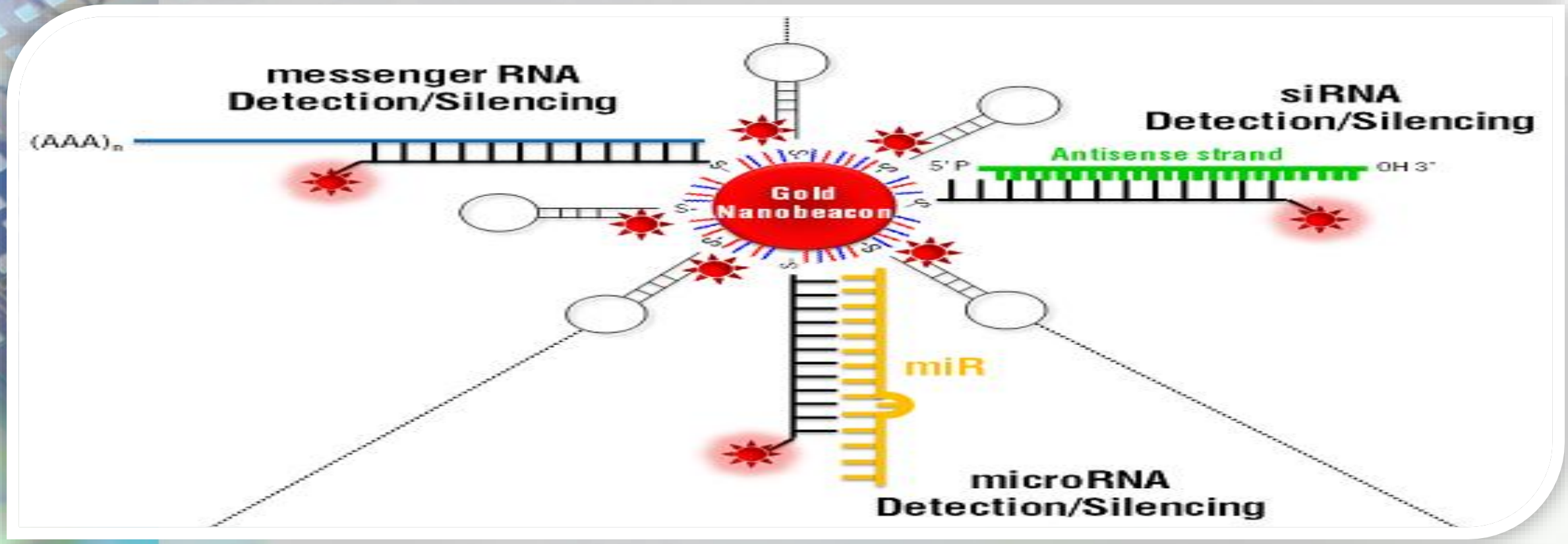
Oligonucleotides

- ✓ Another uses small molecules of RNA called siRNA.(4)
- ✓ To signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene.(4)
- ✓ Disrupting translation of the faulty mRNA.(4)



Gold nanomaterials

- ✓ Colloidal gold is a suspension consisting of sub-micron gold nanoparticles (AuNPs).(17)
- ✓ Variety of technologies including microscopy, electronics, diagnostics, and therapeutics.(17)

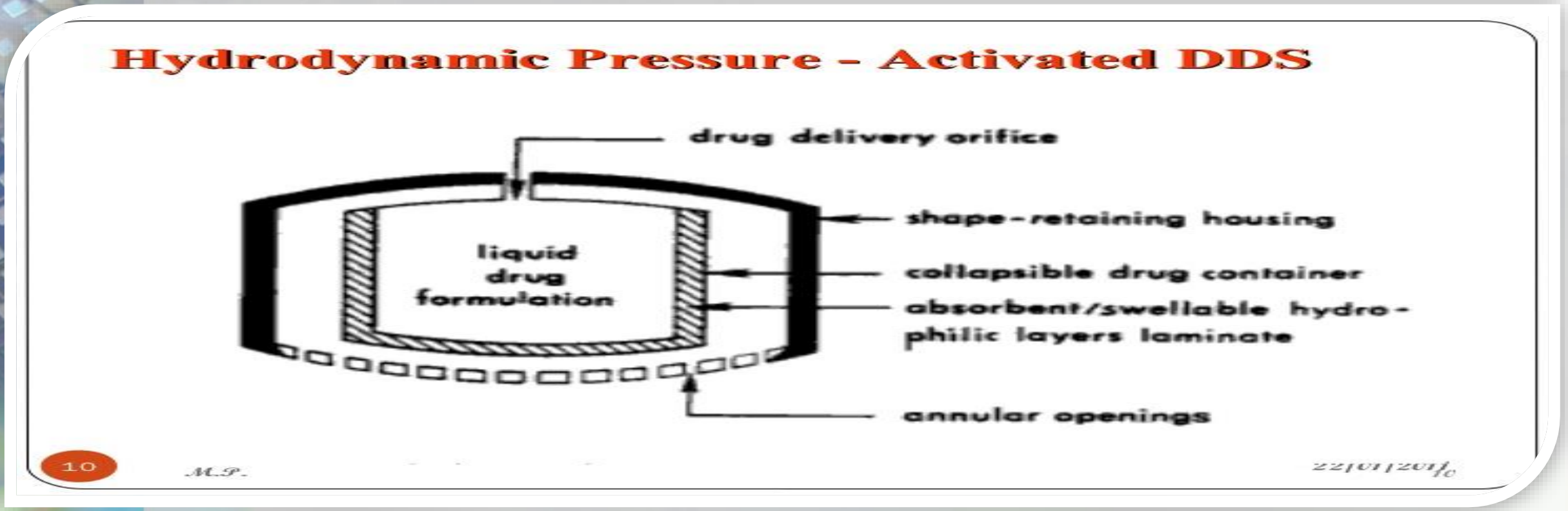


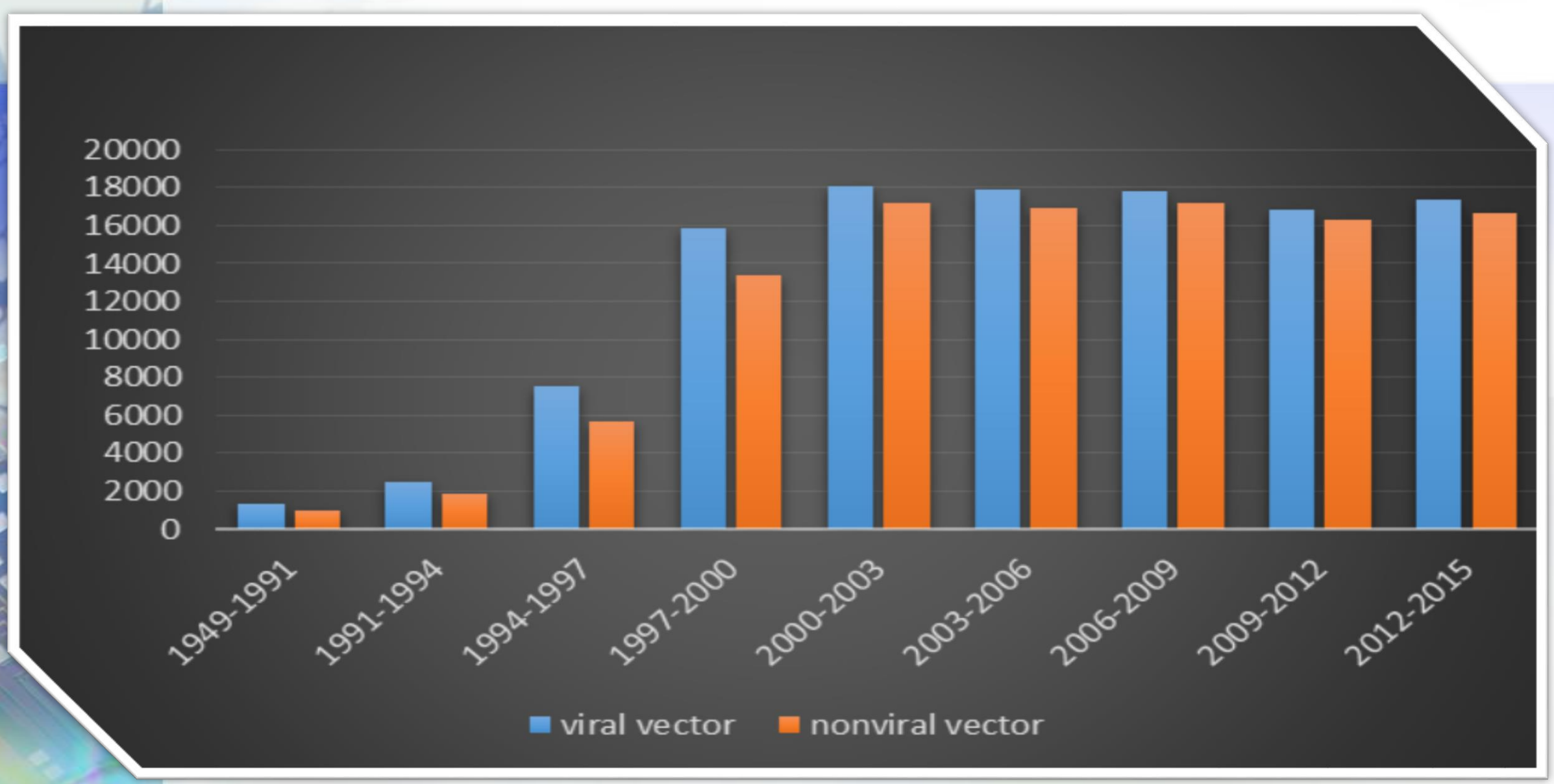
Hydrodynamic

- ✓ A broadly used procedure for DNA and RNA delivery in rodents.(20)
- ✓ Serving as a powerful tool for gene/protein drug discovery.(4)
- ✓ Gene function **analysis**, **target validation**, and **identification** of elements in regulating gene expression.(20)
- ✓ Delivery of a variety of molecules including circular DNA(4)

Hydrodynamic

- ✓ HD through mouse tail veins delivers the gene of interest most effectively to the mouse liver.(4)
- ✓ Successful HD has been achieved in the kidneys, skeletal muscle, myocardium, hepatocellular carcinoma, and brain tumor.(4)





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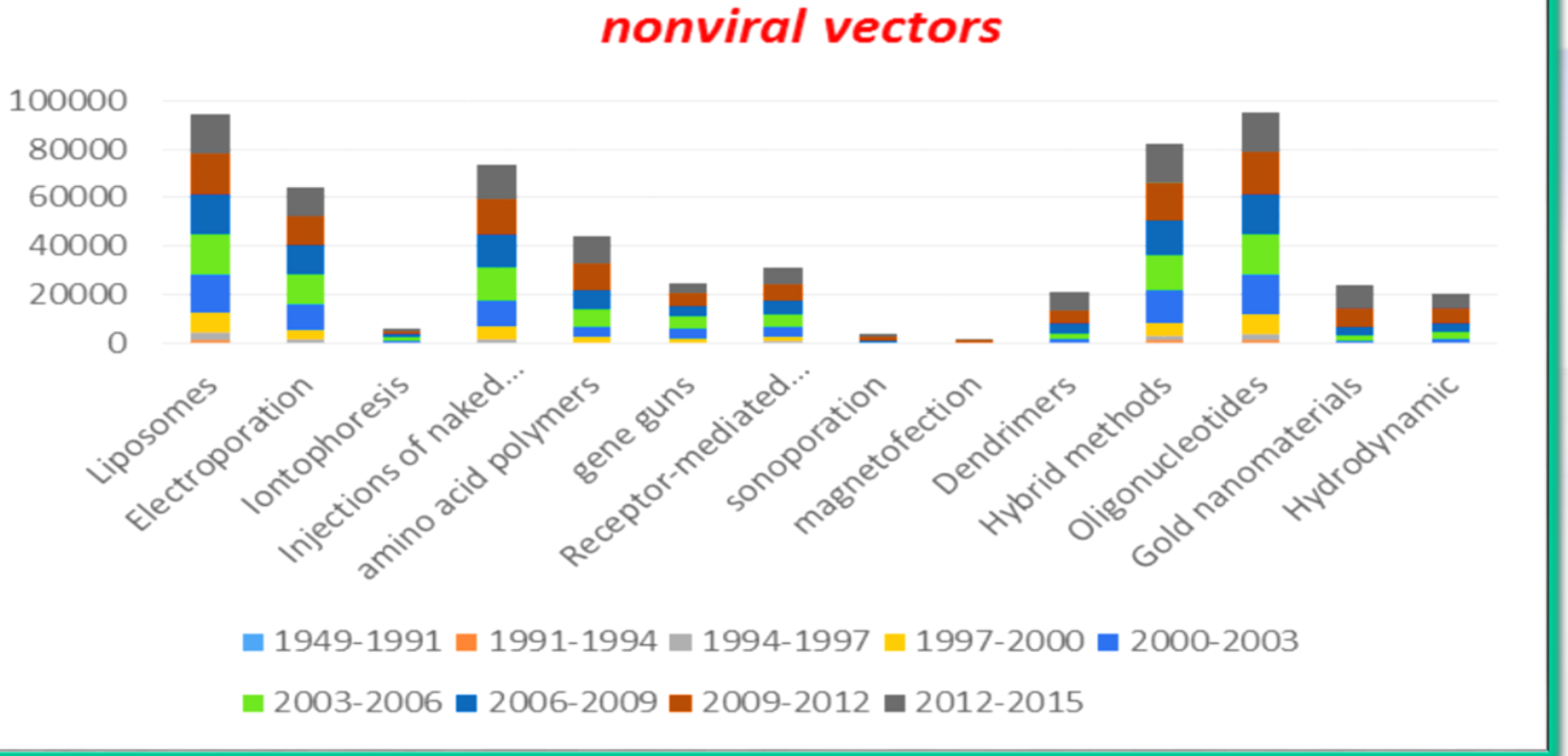

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Thanks for your attention